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EFFECTS OF ISOSMOTIC CONCENTRATIONS OF INORGANIC AND ORGANIC SUBSTRATES ON ENTRY OF WATER INTO CORN ROOTS

H. E. HAYWARD AND WINIFRED B. SPURR[1]

Introduction

The entry of water into plant roots is conditioned by several factors. The metabolic status of the root with respect to carbohydrate reserves, the biochemical changes incident to the entry of solutes from the soil solution, the anatomical differences in roots due to differential rates of growth, and the specific or genetic differences in roots—these all operate to accelerate or retard intake of water (**5, 6, 22**). Soil factors which influence rate of entry of water include the water content of the soil, soil aeration, and the amount of salt in the soil solution (**23**). The force against which the plant roots must act to absorb water is conditioned in part by the osmotic pressure of the solution and by the "capillary" forces of the soil particles (**1**).

Studies of the salt tolerance of a number of plants in sand and water cultures indicate that, in general, the osmotic pressure of the substrate is the most important factor in restricting growth and development under saline conditions. It has also been shown that the toxic effect of specific ions operates in this connection.

Most of the quantitative studies of water intake by intact roots have been made with substrates of relatively low osmotic pressure—tap water or a nutrient culture solution. GREGORY and WOODFORD (**4**) report a few preliminary experiments on water intake which showed extreme variability. HÖHN (**9**), SIERP and BREWIG (**19**), ROSENE (**17**), and HAYWARD, BLAIR, and SKALING (**5**) have made quantitative studies which indicate that maximum rates of water intake occur in the basal regions of young roots, 60–100 mm. from the root tip, rather than in the apical zone immediately above the root cap.

A few studies have reported quantitative data on the rate of water intake by roots as affected by the osmotic pressure of the substrate. ROSENE (**18**), using attached roots of *Allium cepa*, found that substrates with osmotic pressures of 4.2–5.7 atmospheres "represented the range of critical concentrations of sucrose and KNO_3 which will balance the internal factors that operate to determine the velocity of water transport across the epidermal boundary of local root regions." At 25° C. and 100% relative humidity, an osmotic pressure of 5.14 atm. stopped absorption in the majority of intact roots. With leaves exposed to constant light and air at 50% relative humidity and 25° C., absorption ceased when the osmotic pressure of the substrate was 6.5 atm.

TAGAWA (**20**), using intact seedlings of *Phaseolus vulgaris*, studied intake of water in substrates of various osmotic pressures. Using sucrose, Knop's solu-

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tion, $CaCl_2$, KCl, and mixtures of the two salts at osmotic pressures ranging from 0.03 to 2.6 atm., he found that the high pressures reduced intake to about 40% of the rate in distilled water. At equal osmotic pressures (2.4 atm.) intake was about the same with sucrose, KCl, and $CaCl_2$, and somewhat higher with Knop's solution. In mixtures of the chloride salts he found that K^+ accelerated and Ca^{++} retarded entry of water.

HAYWARD and SPURR (**6**) used NaCl added to a nutrient solution to produce osmotic pressures of 2.8 and 4.8 atm. As compared with roots in a control substrate (0.8 atm.), the decrease in rate in the zone of most rapid intake was 64% in the 2.8 atm. and 82% in the 4.8 atm. substrate.

It has been stated that water is equally available to the plant roots from field capacity to the permanent wilting percentage, 0–15 atm. of soil moisture tension (**7, 21**). Since the osmotic pressure of the substrate is an important factor in determining the rate of water entry into roots, more quantitative data are needed to show to what extent specific ions or salts commonly occurring in the soil solution in saline areas influence the entry and at what pressure there is cessation of intake. In these studies, NaCl, Na_2SO_4, and $CaCl_2$ were used as single salts added to the nutrient solution; and, to avoid the possibility of toxic action due to salts, solutions of mannitol and sucrose isosmotic with those of saline substrates were also tested.

Procedure

The potometric device used has been described (**5**). It can be adjusted at any point on the root except where laterals diverge and is adapted to use in substrates of high osmotic pressure, since the test root is submerged in a large volume of solution (33 liters), which eliminates the possibility of significant changes in osmotic pressure due to the intake of solutes. The tests were run under constant conditions of temperature, 72° F. ± 1°, and 70% ± 5% relative humidity. The light was supplied by overhead illumination with Westinghouse white fluorescent tubes. The solutions were continuously aerated with carbon-pipe aerators.

As in earlier studies (**5, 6**), Mexican June corn was used. The test plants were grown in the greenhouse in nutrient solution until they attained the desired size, 10–12 inches tall with five or six expanded leaves and a cycle of adventitious roots exceeding 12 cm. in length. Potometers were attached at root levels 6 and 10 cm. from the root tip, since the 4-cm. zone between these levels has been shown to be the region of most rapid water intake (**6**). Each trial was run for $5\frac{1}{2}$ or 6 hours, with potometric readings at hourly or half-hourly intervals—depending upon the rate of entry. The first readings were not made until the root had been in the substrate for a minimum of 30 minutes, to avoid possible transference reactions (**2, 15, 20**) or initial ionic effects (**13**).

In addition to the base nutrient solution (0.8 atm. osmotic pressure) identical with that in which the plants were grown, saline substrates of two higher osmotic pressures were used (2.8 and 4.8 atm.). The tests with organic solutions were run at 0.8, 2.8, and 4.8 atm. with no added nutrient salts. A few trials were made with a sucrose substrate at 6.8 atm. The freezing-point depression method was employed to determine the osmotic pressures of the substrates, and variations from the theoretical value did not exceed 0.2 atm. in any trial.

Investigation

Inorganic substrates

The rates of entry of water for intact roots in inorganic substrates and those obtained with roots in the base nutrient solution are shown in table 1 and figure 1. The rate of intake was slightly higher at the 10-cm. root level than at the 6-cm. level in all cases except when $CaCl_2$ was the added salt; but the differences in rate at the two levels are significant only in the control substrate. Differences in rate of intake in the three saline substrates at isosmotic pressures are not significant at either the 2.8- or 4.8-atm. levels. This is true when the comparisons are made between rates at either root level (6 or 10 cm.) or between the average mean rates of the two levels.

In all three saline solutions, the difference in rate of water intake between substrates at 0.8, 2.8, and 4.8 atm. osmotic pressure was highly significant when the average mean rate of the individual roots was used as a basis of comparison. At the 6-cm. level there was less difference in rate between the base nutrient and the saline substrates at 2.8 atm. than at the 10-cm. level. In the latter, all differences were highly significant; but at the 6-cm. level, differences in rate between the base nutrient and the $CaCl_2$ substrates at 2.8 atm. were not significant.

Organic substrates

To eliminate the possibility of toxic effects due to salts on the rate of entry of water, experiments were set up using mannitol and sucrose as the solutes. In both cases the substrates were adjusted to 0.8, 2.8, and 4.8 atm. osmotic pressure, and an additional one was run with sucrose at 6.8 atm. to determine the approximate osmotic pressure at which intake of water would stop under the experimental conditions of these studies.

The rates of water intake for intact roots in organic substrates are given in table 2 and figure 2. In all cases the rate was greater at the 10-cm. level than the 6-cm. level; but the differences were not significant, except at 0.8 atm. osmotic pressure, in which results for the organic substrates agreed closely with those of the base nutrient solution. Using the average of the mean water intake for the individual roots, highly significant differences were found in the rates at 0.8, 2.8, and 4.8 atm. osmotic pressure, regardless of the organic substrate used. A comparison of the rates of entry for mannitol and sucrose at isosmotic pressures shows no significant differences in water intake at 0.8 and 4.8 atm. but a significant difference at 2.8 atm. No explanation is advanced for this exception to the relationships determined with all other substrates at this osmotic pressure, and for all substrates, including sucrose at the 0.8- and 4.8-atm. levels.

A few roots were tested in a sucrose solution at 6.8 atm. osmotic pressure. No intake was observed; in fact, a very slight outward movement was noted. Since this result indicated that the critical concentration which would stop intake of water under these experimental conditions was an osmotic pressure of slightly less than 6.8 atm., the pressure of the sap of corn roots grown in the base nutrient solution was determined by a method[2] suggested by Hoagland (8). A value of 5.7 atm. was obtained using sap from roots taken in the morn-

[2] Roots rinsed quickly in distilled water, centrifuged at a r.p.m. to produce a force 40–45 × gravity, frozen rapidly at −15° C., thawed, the sap expressed at 8000 pounds pressure per sq. inch, and freezing-point depression determined.

TABLE 1

MEAN RATE OF ENTRY OF WATER FOR ROOTS IN BASE NUTRIENT AND INORGANIC SUBSTRATES EXPRESSED AS MM.^{3}/MM.^{2}/HR.

ROOT LEVEL (DISTANCE FROM ROOT TIP)	SUBSTRATES AND OSMOTIC PRESSURE						
	Base nutrient	B.n.+Na_2SO_4		B.n.+NaCl		B.n.+$CaCl_2$	
	0.8 atm.	2.8 atm.	4.8 atm.	2.8 atm.	4.8 atm.	2.8 atm.	4.8 atm.
10 cm.	0.243 (30)*	0.140 (16)	0.052 (19)	0.134 (26)	0.044 (18)	0.118 (28)	0.041 (17)
6 cm.	0.164 (30)	0.130 (18)	0.037 (18)	0.115 (28)	0.039 (20)	0.134 (26)	0.033 (17)
Av. 10 cm.+6 cm.†	0.204 (60)	0.135 (32)	0.042 (34)	0.123 (52)	0.041 (34)	0.127 (52)	0.036 (32)

* Figures in parentheses indicate number of tests run. In some cases, the number shown for the average rate is less than the sum of the tests for the two root levels, since only paired observations were used in computing average rate and occasionally one of the pairs is missing.

† Average of mean water intake for individual roots.

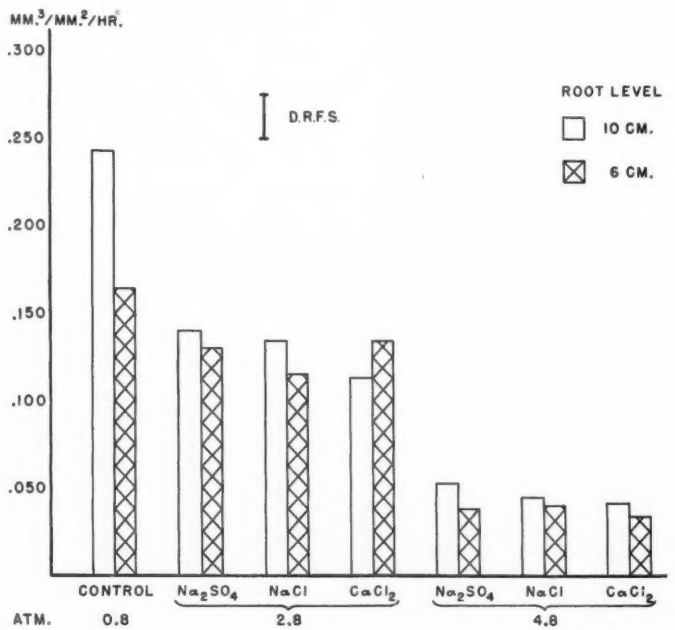

FIG. 1.—Effect of inorganic substrates on rate of entry of water into adventitious roots of corn at 10- and 6-cm. levels. Sodium sulphate, sodium chloride, and calcium chloride added to base nutrient solution (0.8 atm.) as single salts to produce isosmotic pressures of 2.8 and 4.8 atm. Difference required for significance (D.R.F.S.) as derived from pooled error variance = 0.0263 at 5% level.

TABLE 2

MEAN RATE OF ENTRY OF WATER FOR ROOTS IN BASE NUTRIENT AND ORGANIC SUBSTRATES EXPRESSED AS MM.^{3}/MM.^{2}/HR.

Root level (distance from root tip)	Substrates and osmotic pressure							
	Base nutrient	Mannitol			Sucrose			
	0.8 atm.	0.8 atm.	2.8 atm.	4.8 atm.	0.8 atm.	2.8 atm.	4.8 atm.	6.8 atm.
10 cm....	0.243 (30)*	0.257 (8)	0.123 (15)	0.057 (8)	0.255 (12)	0.101 (18)	0.045 (9)	−0.002 (3)
6 cm....	0.164 (30)	0.157 (8)	0.116 (14)	0.030 (7)	0.174 (10)	0.075 (20)	0.035 (9)	0.001 (3)
Av. 10 cm.+6 cm.†	0.204 (60)	0.207 (16)	0.122 (28)	0.042 (14)	0.218 (20)	0.089 (36)	0.040 (18)	−0.001 (6)

* Figures in parentheses indicate number of roots tested. In some cases, the number shown for the average rate is less than the sum of the tests for the two root levels, since only paired observations were used in computing average rate and occasionally one of the pairs is missing.

† Average of mean water intake for individual roots.

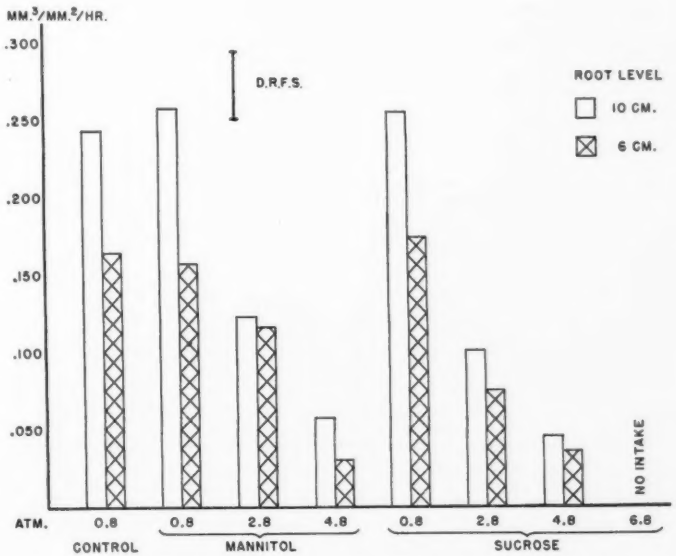

FIG. 2.—Effect of organic substrates on rate of entry of water into adventitious roots of corn at 10- and 6-cm. root levels. Mannitol and sucrose added to tap water to produce desired osmotic pressures. Difference required for significance (D.R.F.S.) as derived from pooled error variance = 0.0452 at 5% level.

ing—the time the experiments were regularly started.

Comparison of inorganic and organic substrates

The average mean rates of water intake for individual roots in inorganic and organic substrates are shown in figure 3. In general, these data indicate no significant difference in rate of intake at a given osmotic pressure of the substrate, regardless of the solute used. At the 0.8-atm. level, the rates for the organic substrates were slightly higher than for the control solution containing nutrient salts. As already noted, an exception occurred at the 2.8-atm. level, where the intake for sucrose was significantly lower than the rates of the other four substrates. Very close agreement in rate was obtained with roots tested in five substrates at 4.8 atm. (fig. 3).

In all substrates, differences in average mean intake between any two levels of osmotic pressure were highly significant. Considering the rate of entry at the 0.8 level as 100%, the reduction in rate at 2.8 atm. ranged from 34% with Na_2SO_4 substrate to 59% with the sucrose. Excluding sucrose, the average reduction for the other four substrates was 38%. At the 4.8-atm. level, the reduction varied from 79% with Na_2SO_4 to 82% with $CaCl_2$ and sucrose, with an average reduction of 81% for the five substrates tested.

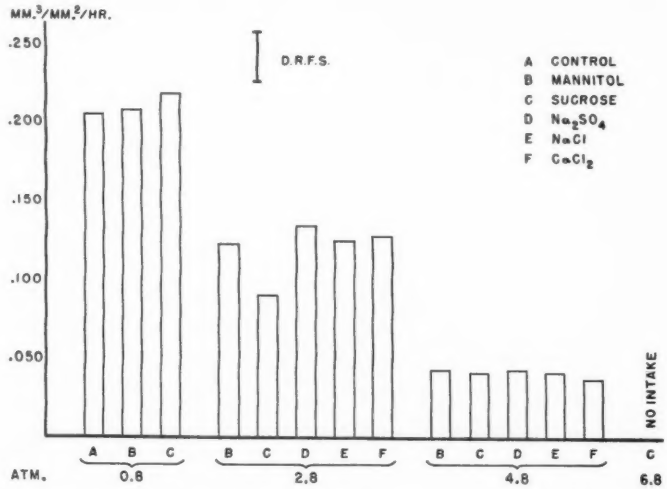

FIG. 3.—Comparison of average mean rate of entry of water for individual roots in organic and inorganic substrates at isosmotic pressures. Difference required for significance (D.R.F.S.) as derived from pooled error variance = 0.0327 at 5% level.

Discussion

The data indicate that total osmotic pressure of the substrate, regardless of the solute involved, is a primary factor limiting entry of water into roots. When uniform corn plants grown under the same nutritional and environmental conditions are transferred to substrates whose osmotic pressures range from 0.8 to 4.8 atm., there is marked reduction in rate of intake with increasing pressure. In

general, the reduction at a given osmotic pressure does not vary significantly due to the kind of solute used. When compared with the 0.8-atm. substrates, the reduction in rate of intake at 4.8 atm. varied only 3% (79–82%), regardless of whether the solute was organic (sucrose or mannitol) or a single salt (Na_2SO_4, NaCl, or $CaCl_2$).

Results in essential agreement with these data have been reported by ROSENE (**18**), who found that the same concentrations of sucrose and KNO_3 would inhibit water transport across the epidermal boundary in *Allium cepa*. TAGAWA (**20**), experimenting with intact seedlings of *Phaseolus vulgaris* in substrates of lower osmotic pressure (approximately 2.4 atm.) than those reported here, found little difference in absorption with isosmotic solutions of sucrose, $CaCl_2$, or KCl. He did, however, obtain marked differences in absorption when mixtures of $CaCl_2$ and KCl were used in ratios of 8:2 and 2:8, respectively. Increase in K^+ accelerated absorption, while increase in Ca^{++} retarded water intake.

It might be assumed that the difference in rate of water entry with mannitol and sucrose substrates is related to a differential permeability of the absorbing cells to these solutes. However, COLLANDER and BÄRLUND (**3**), studying the uptake of sugar by *Chára* from dilute and more concentrated solutions (0.25–6.15 atm.), found that no appreciable amounts of sucrose, glucose, or mannitol were taken up by *Chara* cells in 48 hours. Using approach-grafted tomatoes to study the intake of water and nutrients by roots, LONG (**11**) found that sucrose reduced water intake approximately 30% more than NaCl at equal osmotic pressures (4.8 atm.). He attributed part of the difference to a greater reduction in the pressure of the NaCl solution (4.15 atm.) than the sucrose substrate (4.5 atm.) during the course of the experimental run, but he pointed out that in absolute amounts more sugar than NaCl was removed by the roots. LONG suggests that the absorbed sugar, being respirable, might "contribute less to the internal osmotic pressure than the salt which remains osmotically active." His results are in line with our data for the experiments run with substrates at 2.8 atm. osmotic pressure but are at variance with those at 4.8 atm., where the difference in rate of intake between the sucrose and mannitol substrates was only 5% and not statistically significant.

The effect of various ions on permeability has been investigated extensively. OSTERHOUT (**14**), using the conductivity method on the tissue of *Laminaria*, found that Na^+ and K^+ increased permeability regardless of the anion with which the cation was paired, while Ca^{++} tended to decrease it. LUCKÉ (**12**) states that decrease in permeability is produced by cations which act in proportion to their valence. On this basis it might be expected that intake of water would be more rapid with a NaCl substrate than with an isosmotic solution of $CaCl_2$. Our data indicate no significant differences with the salts used. This may be related to the observation of OSTERHOUT (**13**) that the inhibiting effect of $CaCl_2$ is not permanent and if exposure is prolonged will be followed by an increase in permeability. Using *Laminaria*, he found that the peak in resistance was reached in 15 minutes and was maintained for about an hour, after which there was a rapid drop. Since our readings were not begun until the plants had been in the substrate for at least 30 minutes, it seems probable that any initial effect of the Ca^{++} ion would not be significant. In fact, examination of the first half-hourly

readings as compared with the rates of intake at subsequent half-hour intervals does not reveal any consistent trend with the $CaCl_2$ substrate. TAGAWA (**20**) found the "transference" reaction to be about the same for sucrose, KCl, and $CaCl_2$ solutions. When intact bean seedlings were placed in the concentrated substrates (2.4 atm.) there was a sudden drop in water absorption, which lasted about 30 minutes, and then intake increased gradually—reaching constant values in at least one hour after transference.

The data obtained by us as to the osmotic pressure at which intake of water ceases are in general agreement with those of ROSENE (**18**), who found it to be 6.5 atm. for *Allium cepa* when the leaves were exposed to constant light and air at 50% relative humidity and 25° C. Our value of approximately 6.8 was obtained under a constant temperature of 22° C. and relative humidity of 70%. KÖHNLEIN (**10**), using decapitated corn plants and a manometric technique, was able to stop exudation by adding sugar solution of 2.5 atm. osmotic pressure. This value is in line with our previously published results for decapitated corn plants, in which the rate of water intake was reduced 56% as compared with intact plants (**6**). On the basis of his critical concentration and our values as to percentage reduction in intake due to decapitation, it would probably require a substrate of approximately 5.7 atm. to inhibit entry of water.

These data have interesting implications in relation to practical problems of irrigation agriculture in saline areas. The range in the "capillary" force with which water is held by soil particles between field capacity and the permanent wilting point is approximately 0.1–15 atm. of tension (**16**). If water intake by corn roots is reduced as much as 80% when the osmotic pressure of the substrate is raised from 0.8 to 4.8 atm., and completely inhibited at 6.8 atm., it is questionable whether water held in the soil with a force of 6.8 atm. is readily available to the plant. Since the moisture content corresponding to such a force lies between the moisture equivalent and the permanent wilting percentage, it would appear that water is not equally available between field capacity and the wilting range, as has been proposed by some investigators (**7, 21**). It therefore seems clear that both the force with which water is held by the soil particles and the amount of soluble salts present in the soil solution are important factors when considering the water available to the plant.

The salt tolerance of a plant may be related in part to the osmotic pressure of the sap that a given species can maintain. This, in turn, is undoubtedly conditioned by its nutritional status. Hence a more complete understanding of the metabolism of the plant as related to the synthesis of carbohydrate reserves, the effect of such reserves on concentration of the sap, and the relation of fertilizer practices to metabolism is indicated.

Summary

The data are interpreted as indicating that the osmotic pressure of the substrate is one of the primary factors in controlling the rate of entry of water into roots. It is also recognized that the character of the solute and toxicity of salts or ions may have a significant bearing on the function of water intake, and further experiments are planned—especially with mixed salts. The conflicting

data regarding the effect of organic substrates are discussed in the light of other work. The results with inorganic substrates indicate that, under the conditions of these experiments, no significant differences in rate of intake occur when isosmotic pressures of Na_2SO_4, NaCl, or $CaCl_2$ are used as single salts.

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CHANGES IN THE ORGANIC-ACID CONTENT OF VALENCIA ORANGES DURING DEVELOPMENT[1]

WALTON B. SINCLAIR AND R. C. RAMSEY

Introduction

It is well known that the free acidity of orange juice decreases and the pH increases during growth and maturation of the fruit. These facts are of practical importance in establishing maturity tests for commercial purposes, but they do not reveal any information about the kinds and amounts of organic acids produced in the fruit with the advance of the season.

In previous studies, SINCLAIR, BARTHOLOMEW, and RAMSEY (9), working largely with mature citrus fruits, have shown that the total acidity of orange juice is due chiefly to citric and malic acids. The concentration of malic acid in different juices varied only slightly, however, as compared with the changes in the citric-acid content. With one exception, the samples studied had a malic-acid content of 1.40–1.77 mg. per milliliter of juice, while the citric-acid content varied from 8.38 to 25.39 mg. per milliliter. It is thus evident that variations in acidity of the orange are due chiefly to changes in the citric-acid concentration. A definite relation was also found between the free-acid—combined-acid balance and the pH of the juice. The concentration of the combined acids in the juice is remarkably uniform; this means that the free-acid concentration is the chief variable.

On the basis of these results, experiments were planned to study the changes in the organic-acid constituents of the fruit of Valencia orange trees during ripening. Such experiments required sampling of the fruit from a given plot of trees at intervals during the growing season and subsequent determination of the pH, the soluble-solids content, the citric- and malic-acid contents, the potentiometric titration curve, and the alkalinity of the ash of the juice of each sample. The size and weight of the fruit, dry matter of the pulp, and the total free acid per fruit were also determined on each sample. From such determinations, the relation of pH to the free acid, and also to the free-acid—combined-acid balance, was studied. The results of these investigations are reported here.

Material and methods

Samples of fruit for this experiment were taken at approximately monthly intervals during the season from October 2, 1943, to May 2, 1944, from a plot of nine Valencia orange trees selected for this study. At the time of the monthly sampling, six average-sized fruits were picked at intervals around the circumference of each tree. The equatorial and longitudinal axes were measured on the fifty-four fruits in each sample, for the estimation of average size and surface area of the fruit. Twenty fruits were taken at random from the sample and weighed, first with and then without peel. Of the twenty peeled fruits, four of average weight were ground in a Waring blender, strained, and made to volume; an aliquot was then titrated to determine the total free acidity per fruit. To determine dry weight, slices of the pulp from the peeled fruits were placed

[1] Paper no. 519, University of California Citrus Experiment Station, Riverside, California.

in aluminum weighing cans and dried in a vacuum oven for 60 hours at 65° C.

The remaining thirty-four fruits of each sample were juiced for the quantitative determinations of the acid constituents of the juice. For these analyses about 300 ml. of centrifuged juice was used. The refractive index was determined with an Abbé refractometer at 27° C. The percentage of soluble solids and the specific gravity were read from a sucrose table. All pH values were determined with a Beckman glass-electrode pH meter at 23° C. For the titrations and the citric-acid analysis, the juice was diluted with distilled water so that 10 ml. was equivalent to 1 ml. of juice.

The free acidity was determined by titrating an aliquot portion of the juice with standard NaOH to the phenolphthalein end point. The inflection point on the (potentiometric) titration curve was taken to represent most accurately the amount of free acidity in the juice. The quantitative determination of citric acid, free and combined, was made on the juice samples by the pentabromacetone method of PUCHER *et al.* (**8**). Since their procedure specifies a quantity of citric acid of 1 to 20 mg., it was necessary to modify their method slightly when juice samples containing over 20 mg. citric acid per milliliter were being studied. The only change necessary was an increase in the amount of standard $AgNO_3$ added in the final steps to measure the bromide ion present.

In the determination of the malic acid present, it was necessary to separate the organic acids from the other constituents in the juice. HARTMANN and HILLIG'S (**6**) method was used for extracting these acids. Ten milliliters of saturated lead acetate was added to 25 ml. of undiluted juice, and the mixture was diluted to 135 ml. with 95% ethyl alcohol. The precipitated lead salts of the organic acids were separated from the solution by centrifuging and were then suspended in approximately 200 ml. of H_2O and heated to boiling. Hydrogen sulphide was bubbled through the mixture until it was saturated, and the lead sulphide then filtered off. The filtrate containing the organic acids was made to a volume of 250 ml., of which 10 ml. was equivalent to 1 ml. juice. The malic acid was determined quantitatively on 10 ml. of this solution by the method of PUCHER *et al.* (**8**).

The amount of combined organic acid in the juice was found experimentally by measuring the alkalinity of the ash from an aliquot of juice. For each sampling, four 50-ml. aliquots of juice were ashed at 450° C. to a white residue. To this ash was added 5 ml. of standard 2 N HCl, and the whole was washed into a 100-ml. volumetric flask. The flask was made to volume with water, and a 10-ml. aliquot was titrated with standard NaOH. The difference in titer of NaOH between the sample and a blank solution of the HCl represents the alkalinity of the ash. The amount of HCl (in milliequivalents) neutralized by the ash is a close approximation of the milliequivalents of combined organic acid in the juice, since these organic salts decompose to carbonates during the ashing.

Results

Changes in free-acid content with fruit growth

The components of fruits are usually expressed as percentages of the fresh or dry weight. Percentages calculated on these terms are satisfactory for most practical purposes, but from a physiological standpoint it is sometimes important to know the amount of a given constit-

uent per fruit. For this reason, growth curves, as shown by the rates of increase in fresh weight of the fruit during the season, have been determined (fig. 1). These curves show almost no growth during the winter months, only slight changes in the pulp weight occurring during December and January, probably because of the rainy season and the comparatively cold weather. Both before and after this period of slow growth, the rate of development was more or less uniform. As would be expected, the increase in weight of the pulp was much greater than that of the peel. Over the 7-month period covered by the samplings, the average weight of the peel, per fruit, increased from 25.3 to 49.2 gm., while that of the pulp increased from 37.7 to 114.5 gm. per fruit (table 1).

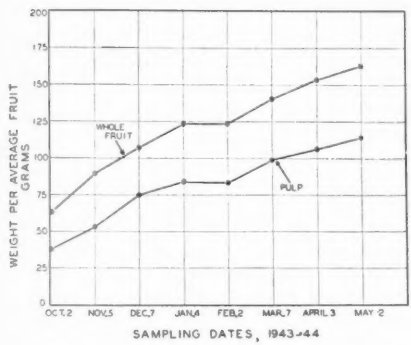

FIG. 1.—Relative weights of whole fruits and pulp of samples of Valencia oranges picked at intervals during season.

The amount of free acid per average fruit was determined on each sample in order to ascertain at what stage of development the organic acids appear in the growing fruit. As shown by the curves in figure 2, the acids increase in the fruit during the early growth period and then remain rather uniform in amount until maturity. The absolute amount of acid (milligrams per fruit) in the samples of these experiments had nearly reached a maximum by December 7. The slight variations after that date are probably due to experimental and sampling errors. The decrease in concentration of acids per milliliter of juice during ripening was evidently caused by the growth of the fruit and the consequent dilution of the acids present; for as the fruit increases in size, the acids must be distributed through an increasing volume of juice. These results are in agreement with those of BIGELOW and GORE (3), who showed that in Navel oranges the total acid per fruit was produced early in the season and varied only slightly in amount during subsequent growth. Similar results were also noted by MCDERMOTT (7).

The total free-acid content per fruit was also determined indirectly by converting the total water content per fruit into its equivalent volume of juice. This can be done if it is assumed that the total water content of the pulp is water of dilution from the juice. In other words, it must be assumed that the acid is uniformly distributed throughout the water content of the pulp. For example, with the April sampling the water content of the pulp averaged 85.04% of the fresh weight of the fruit (106.1 gm.), or 90.2 gm. H_2O per fruit. From the refractive index, the water fraction of the juice was found to be 86.93% and the specific gravity, 1.0528. In 1 ml. of juice there would be $0.8693 \times 1.0528 = 0.915$ gm. H_2O. Therefore, 90.2/0.915 would represent the milliliters of juice (98.6) per fruit. Since the free acidity (16.45 mg.) per milliliter of juice is known from the potentiometric titration, the free acid (as citric) per fruit can be found:

$$98.6 \times 16.45 = 1621 \text{ mg.}$$

TABLE 1

CHANGES IN SIZE AND IN TOTAL FREE ACIDITY OF VALENCIA ORANGE FRUITS DURING GROWTH

Sampling date	Av. dimensions of fruits (cm.)		Av. weight per fruit (gm.)		Dry matter in pulp (%)	Total free acidity (as citric acid) per fruit (mg.)	
	Longitudinal axis	Equatorial axis	Peel and pulp	Pulp only		Experimental determination	Calculated
Oct. 2, 1943	5.23	4.92	63.0	37.7	14.29	1090	1371
Nov. 5, 1943	5.86	5.60	89.0	52.8	13.91	1085	1436
Dec. 7, 1943	6.16	5.95	107.2	75.0	13.20	1416	1689
Jan. 4, 1944	6.43	6.19	122.8	84.5	13.40	1443	1740
Feb. 2, 1944	6.49	6.25	122.2	82.1	13.64	1371	1608
Mar. 7, 1944	6.69	6.42	140.1	98.2	14.41	1388	1736
Apr. 3, 1944	6.92	6.53	153.0	106.1	14.96	1455	1621
May 2, 1944	7.10	6.71	163.7	114.5	15.08	1493	1710

The free acidity value (per fruit) calculated by this method is considerably higher than the actual experimental results. The assumption that the acid is uniformly distributed in the total water of the pulp is undoubtedly the cause of the high results. Not all the moisture in the fruit is extracted as juice, and acid determinations on aliquot portions of the juice produce high results when calculations are made on the basis of the total water content per fruit. The fact that the amount of soluble solids varies considerably in the juice extracted from different portions of the pulp is strong evidence that the acid is not uniformly distributed in the fruit pulp. However (fig. 2), the plotted values for the calculated results of free acidity per fruit give a curve similar to the experimental results.

Citric- and malic-acid contents of fruits picked at intervals during season

The citric- and malic-acid contents of orange juice at different stages of fruit maturity are shown graphically in figure 3. During the period studied (October 2, 1943, to May 2, 1944), the concentration of citric acid and citrate decreased from 39.88 to 17.21 mg. per milliliter of juice, but the concentration of malic acid and malate showed only slight variation. However, the malic acid showed an increase during the season, if the calculations were made on the basis of the total water content per fruit (fig. 2, curve 4). It must be emphasized that curve 4 was drawn from calculated values and was not experimentally determined. These calculated values have been found to be slightly higher than the experimental results as shown for citric

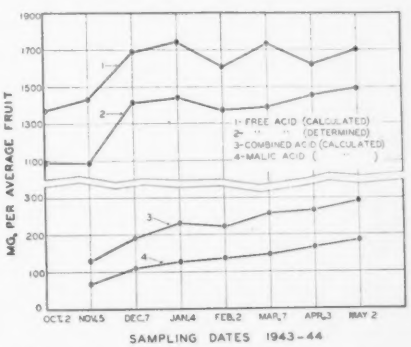

FIG. 2.—Changes in various acid constituents per fruit during fruit development.

acid. The important thing, however, is that the total malic-acid content per fruit increased during the growth period, and the concentration of malic acid, in milligrams per milliliter of juice, showed little change.

The free-acid curve (fig. 3), as determined by the potentiometric titration, is lower on the graph but runs parallel with the citric-acid and citrate curve. It follows, therefore, that the decrease in the concentration of the free acid of

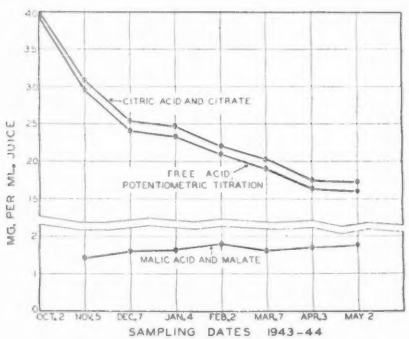
CITRIC ACID AND CITRATE
FREE ACID POTENTIOMETRIC TITRATION
MALIC ACID AND MALATE
MG. PER ML. JUICE
OCT.2 NOV.5 DEC.7 JAN.4 FEB.2 MAR.7 APR.3 MAY 2
SAMPLING DATES 1943-44

FIG. 3.—Changes in concentrations of various acid constituents in juice during maturation of fruit.

orange juice during ripening is due chiefly to the decrease in citric acid. This result is quite different from that given in figure 2, curve 2, which shows that after the citric acid reached a maximum in the early stage of fruit development, it varied only slightly during the remainder of the ripening period.

RELATION OF FREE ACID TO COMBINED ACIDS IN FRUITS AT DIFFERENT STAGES OF MATURITY

As previously noted, the concentration of free acid decreased with growth and maturity of the fruit. This is shown in table 2, in which the phenolphthalein and potentiometric titration values and the citric-acid determinations (pentabromacetone method) decreased with each successive sampling of the fruit. The concentration of combined acids in the juice, however, showed only slight variations over the entire period studied.

The results for the combined acids, as determined by the two very different methods (table 2), are in close agreement. The "calculated" values were determined as the difference between the milliequivalents of free acid and the sum of the milliequivalents of total citric and total malic acids of the samples. The direct determination of combined acids was based on the fact that the salts of organic acids decompose to carbonate salts during ashing. The close agreement between the results of the two methods indicates the validity of the quantitative measurements of total citric and total malic acids. The malic acid and the alkalinity of the ash were not determined on the first sampling of fruit, so there are no combined-acid values for that sample.

Since the concentration of the combined acids did not change appreciably as the fruit grew, it is evident that the absolute quantity per fruit increased gradually, as shown in figure 2. The conversion of the concentration of combined acids from a juice basis (milligrams per milliliter) to a fruit basis (milligrams per fruit) was made according to the method explained for the similar conversion of total free acidity. These calculated results are slightly higher than the actual amount of combined acids per fruit, but they show that the combined acids, unlike the free acid, continued to increase in the fruit during growth and ripening. On that basis, it can be assumed that more inorganic minerals were made available for salt formation, since the cation concentration, and not the acid radical, is the

TABLE 2

CHANGES IN CONCENTRATION OF ACID CONSTITUENTS OF ORANGE JUICE DURING GROWTH OF FRUIT

Sampling date	Soluble solids	pH	Total free acid (as citric)			Citric acid (pentabromacetone method)		Malic acid		Combined acids (as citric)				Acid radical in free form
			Phenolphthalein titration	Potentiometric titration						Calculated*		Determined from alkalinity of ash		
	(%)		(mg./ml.)	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	(%)
Oct. 2, 1943	9.91	2.72	39.24	39.29	0.614	39.88	0.623	†	†	†	†	†	†	†
Nov. 5, 1943	9.91	2.81	30.47	29.58	.462	30.84	.482	1.40	0.021	2.62	0.041	2.75	0.043	91.8
Dec. 7, 1943	10.57	2.88	24.44	24.18	.378	25.39	.397	1.58	.024	2.75	.043	2.74	.043	89.8
Jan. 4, 1944	10.83	2.91	23.69	23.38	.365	24.72	.386	1.62	.024	2.88	.045	2.68	.042	89.1
Feb. 2, 1944	11.37	2.95	21.32	21.07	.329	22.15	.346	1.78	.027	2.81	.044	3.01	.047	88.2
Mar. 7, 1944	12.37	3.03	19.34	19.02	.297	20.28	.317	1.59	.024	2.81	.044	2.92	.046	87.1
Apr. 3, 1944	13.07	3.07	16.66	16.45	.257	17.51	.274	1.68	.025	2.69	.042	2.89	.045	86.0
May 2, 1944	13.12	3.11	16.35	16.11	0.252	17.21	0.269	1.75	0.026	2.75	0.043	2.83	0.044	85.4

* Calculated as difference between milliequivalents of free acid and sum of milliequivalents of total citric and malic acids present.

† Data not determined.

limiting factor in the formation of organic salts.

The two curves of figure 4 illustrate the relation of pH to the concentration of free acid and also to the free-acid—combined-acid balance. For curve A, the term "free acid" indicates the percentage of the total-acid radical present in the juice as free acid. In equation form, with acidity values expressed in milliequivalents,

$$\frac{\text{Free acidity}}{\text{Total citric acid} + \text{total malic acid}} \times 100 = \text{percentage of free acid.}$$

increase in pH during ripening to changes in the ratio of acid salt to free acid. This change in ratio is due mostly to the change in free acid, for BIOLETTI *et al.* (4) have noted that the rate of increase of cream of tartar during ripening could not account for the decrease in free acidity.

Curve B (fig. 4) shows the relation between pH and the free acid of the juice, calculated as percentage of fresh weight. The values forming this curve were determined on fruit samples picked from trees in the same locality. The variables were thus reduced to a minimum. If the

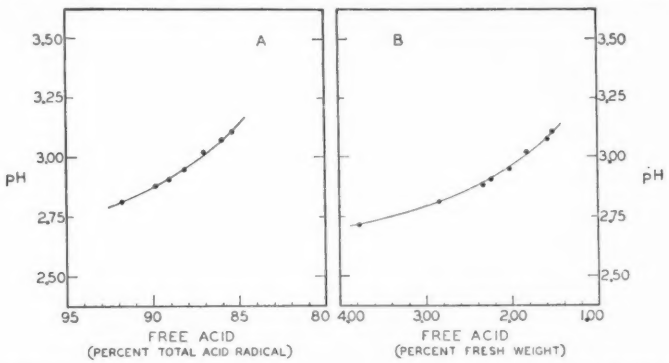

FIG. 4.—Relation of pH to free acid (*A*) expressed as percentage of total-acid radical of juice, and (*B*) calculated as percentage of fresh weight. Points on curves represent samples picked between November 5, 1943, and May 2, 1944, for curve *A;* and between October 2, 1943, and May 2, 1944, for curve *B*.

As the fruit matured, the percentage of acid in the free state decreased and the pH of the juice increased. The rise in pH was the result of the change in the value log (salt/acid), since the acid concentration decreased during ripening and the salt (combined-acid) concentration remained nearly constant.

AMERINE and WINKLER (1) have shown that the amount of tartrates present as free acid in grapes was reduced from a range of 50–80% to a range of 10–20% during growth and ripening of the fruit. They attributed the gradual

pH is plotted against the percentage of acid in the juice of fruits grown on different rootstocks and in different locations, the variations are more pronounced. To illustrate with an actual but extreme example (2), juice from Washington Navel oranges grown on Rough-lemon rootstock showed 0.80% total acids (in terms of citric acid), with a pH of 3.52, while juice from Navel oranges grown on Trifoliate orange showed 1.21% total acids, with a pH of 3.46. Although there was a 34% difference in the total acidity of these two juice samples, the pH values

differed only slightly. These two samples of Navel oranges were picked on the same day (March 3, 1942), from two different blocks in the same grove. It is very probable that several variables produce the change in the relation of pH to the percentage of free acid (fresh weight of juice).

The following equation (5) is approximate for calculating the pH of weak acids:

$$pH = pK_a + \log \frac{salt}{acid},$$

where the term "salt" indicates that of the organic acid present. Since the citric- and malic-acid systems in orange juice can be classed as weak acids, this equation may be applied. Over the concentration changes occurring in the juice, the variation pK_a is very slight; it may therefore be assumed to be a constant in this discussion. That leaves the factor log (salt/acid) as the only one that may vary the pH.

The dilution effect of the water in the juice is undoubtedly an important variable. This water content may vary considerably and thus cause variations in the concentration of the acid although the absolute amount of acid present remains constant. The variations in water content do not change the value of log (salt/acid), however, as the salt and acid would be diluted or concentrated in the same proportions. The pH of the juice would therefore remain constant, but the concentration of the acid could vary over a wide range.

The opposite may also occur; that is, the pH may vary while the acid concentration remains unchanged. This effect would be caused by the variation in value of the factor log (salt/acid). If the acid concentration remains constant, then the numerator, or salt concentration, must cause the pH variation. It is probable that with fruit from many different trees and groves the amount of salt in the juice would vary enough to produce a significant variation in pH.

With a system such as orange juice, a combination of variables very likely produces the wide variation in the relation of pH to the percentage of acidity on a fresh-weight basis. For example, the extent of dilution could result in a decrease in concentration of the acid, and at the same time a change in the absolute amount of salt might change the pH in the direction of increased acidity. Other factors probably enter into the relation, but the two variables just discussed are considered the most important.

Summary

The maximum amount of free acid in Valencia orange fruits was found to develop early in the season and to change very little from that time on. The concentration of free acids in the juice (milligrams per milliliter), however, lessens considerably during fruit development. This decrease in free acidity, with the corresponding increase in pH, was due chiefly to the decrease in concentration of free citric acid. Although the malic-acid concentration in the juice (milligrams per milliliter) stayed nearly uniform during the season, the actual amount in the fruit increased. The concentration of combined acids remained nearly uniform in the fruit, but the absolute amount per fruit increased. The amounts of combined acids determined from the alkalinity of the ash were in agreement with the values determined from the difference between the total-

and free-acid radicals. During ripening, the changes in pH of the juice were definitely related to changes in percentages of the total-acid radical in the free form. A similar relation was noted between pH and the percentage of free acid expressed on a fresh-weight basis.

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GROWTH, RUBBER STORAGE, AND SEED PRODUCTION BY GUAYULE AS AFFECTED BY BORON SUPPLY

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Introduction

Crop plants vary with respect to the optimum amount of boron they require for vegetative growth and reproduction and with respect to their ability to tolerate wide variations in their boron supply (**2**). Symptoms of boron toxicity are exhibited in the vegetative growth of such crops as lettuce and kidney bean when these are grown in sand culture and supplied with a nutrient containing 0.8–1.0 p.p.m. of boron, while others—such as celery, sugar beet, and alfalfa—grow vigorously when supplied with several times this amount (**2**). Seed production by a number of crop plants, particularly legumes, is known to be greatly reduced when the plants are grown with an inadequate boron supply, and their vegetative growth is also decreased by boron starvation (**7**).

Whereas most crop plants which have been studied as to their boron requirement are mesophytic, guayule, indigenous to dry regions, is a xerophytic type of plant. Also, the boron content of the soil and irrigation waters varies widely in the different localities within the United States which are suited to the cultivation of guayule (**8**). Experiments were undertaken to observe some of the responses that result when this plant is subjected to different levels of boron

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nutrition and to determine its tolerance toward variation in the boron supply.

METHODS.—In a preliminary experiment, 100 uniform transplants (variety 593) of guayule were selected from a large number obtained from a nursery near Salinas, California. They were planted in carefully washed, grade 8 Berkley quartz sand contained in glazed earthenware crocks. The crocks were drained by means of a hole in the bottom, which was covered with loosely packed glass wool to prevent loss of sand. The behavior of guayule plants grown in a boron-free nutrient indicated that no effective amount of boron was derived from either the quartz sand or the crock. The plants were divided into two equal lots. One lot was supplied daily with nutrient made with distilled water and containing 8.75 ml. of 0.5 molecular KH_2PO_4 and $MgSO_4$ per liter, respectively, and 12.15 ml. of 0.5 mol. $Ca(NO_3)_2$ per liter. Iron, manganese, copper, and zinc were also supplied to the plants in concentrations of 0.5 p.p.m. of nutrient solution. The remaining plants were supplied with the same solution but containing sufficient boric acid to make a concentration of 0.5 p.p.m. of boron. After growing for 6 weeks under greenhouse conditions, the boron-deficient plants were divided into five groups, and during the following 8 weeks they were given the same basic nutrient but containing 0.0, 0.25, 0.5, 1.0, and 4.0 p.p.m. boron, respectively.

In a subsequent experiment, seeds were germinated, in September, 1942, in a thin layer of sand which was spread over soil. When approximately 2 inches tall, selected plants were dug and the soil carefully washed from their roots. The seedlings were then planted individually in 2-gallon glazed earthenware crocks containing a mixture of coarse sand and fine gravel which had been washed carefully four times previous to use in order to eliminate soil particles. The crocks were rounded on the lower side and drained by means of a hole approximately 1 inch in diameter located in the center of the bottom. A screen covered with asphalt paint was placed over the hole to prevent loss of sand gravel. The crocks were arranged in a greenhouse in such a way that seven treatments could be applied as randomized blocks. A basic nutrient was made up of 17.5 milliequivalents of $Ca(NO_3)_2$, 2.6 me. of KH_2PO_4, 2.6 me. of $MgSO_4$, and 0.3, 0.25, 1.0, and 1.0 mg. of iron, manganese, copper, and zinc, respectively, per liter of distilled water. Seven boron levels were prepared by adding to aliquots of the basic nutrient sufficient boric acid to make solutions containing 0.0, 0.01, 0.1, 0.5, 2.0, 5.0, and 10.0 p.p.m. of boron. These solutions were applied daily in amounts sufficient to flush the cultures. Distilled water was added at intervals during the day to maintain optimum moisture conditions.

The plants were grown under greenhouse conditions until April, 1943, when they were moved outdoors. At this time it was necessary, in making up the nutrient solution, to substitute tap water which contained 0.004 p.p.m. of boron, thus increasing the amount of boron at each nutrient level by this amount. The plants were grown outdoors until October, when they were again returned to a greenhouse and grown with the same nutrient treatments, using tap water and under a controlled day temperature of 55°–65° and a night temperature of 35°–45° F.

Seeds were collected at approximately weekly intervals during the summer (June 1 to September 1) and fall (September 1 to October 16) of 1943. These

were cleaned, then sorted into three sizes by separating those that failed to pass an 8-mesh per inch screen, those that passed the 8-mesh but failed to pass a 10-mesh, and those that passed a 10-mesh screen. The seeds were stored at room temperature for several months in sealed paper envelopes until they were tested for germination as previously described (6). In March, 1944, the plants were harvested and their fresh weights determined. The leaves were removed, and the stems and roots were cut into small pieces and dried for 24 hours in a well-ventilated oven at 60° C. Rubber and resin determinations of stems and roots were made by the SPENCE and CALDWELL method (10). Chemical determinations of the amount of boron in the stems and roots and in the seeds were made by the WILCOX (14) and the BERGER and TRUOG (1) methods.

At the time of final harvest, eight plants from each of four different levels of boron supply (0.004, 0.1, 0.5, and 10.0 p.p.m.) were sampled for the purpose of anatomical study. A segment approximately 1 cm. in length was secured from the base of the main stem of each plant, all pieces being taken from the lowermost 3 cm. of the stem in order that the sections should be comparable. The lower limit of this region was identified in part by the crowded leaf scars just above the cotyledonary node but mainly by the appearance of medullary resin canals, which are present in the stem but not in the hypocotyl (4). The manner of preparing slides and determining tissue areas has been described earlier (13). Data were obtained for the cross-sectional area of the wood, consisting of the pith and xylem, and of the bark, consisting of those tissues external to the xylem but internal to the cork. Within the bark, the major rubber-storing portion of the stem, fiber tissue decreases the potential storage area. Measurements of the area occupied by fiber were therefore made.

In an experiment designed to illustrate the toxic effects of relatively high concentrations of boron, the same cultural methods were used as previously described. Field-grown seedlings were transplanted to a sand-gravel mixture and supplied with nutrient containing 8.75 ml. of 0.5 mol. KH_2PO_4 and $MgSO_4$ per liter, respectively, and 12.15 ml. of 0.5 mol. $Ca(NO_3)_2$ per liter. The basic nutrient solution also contained 0.5 p.p.m. of zinc, iron, copper, and manganese, respectively. Boric acid was added to the nutrient to obtain solutions with boron concentrations of 0.0, 0.5, 2.0, 5.0, and 10.0 p.p.m. These were applied respectively to five groups, each consisting of eight selected plants grown in a greenhouse. Since no toxicity symptoms were apparent, after 3 weeks of treatment the amounts of boron were increased in the case of the plants of two groups, as indicated in table 1. At the end of the following 4-week period all plants were harvested, and the weight of dead leaves per plant and also the boron content of green leaves and of the stems and roots were determined.

TABLE 1

LEAF GROWTH AND BORON CONTENT OF STEMS AND LEAVES OF GUAYULE SUPPLIED NUTRIENT CONTAINING DIFFERENT LEVELS OF BORON

BORON IN NUTRIENT SOLUTION (P.P.M.)		DEAD LEAVES (%)	BORON IN DRY MATTER (P.P.M.)	
First 3 weeks	Following 4 weeks		Stems	Green leaves
0.0	0.0	.....	22	22
0.5	0.5	8.0	25	78
10.0	10.0	15.4	62	517
2.0	20.0	23.2	82	817
5.0	40.0	37.5	171	1189

Results

RESPONSE TO LACK OF BORON.—In the preliminary experiment designed to show responses to the lack of boron, the guayule transplants exhibited characteristic boron-deficiency symptoms after being supplied with a boron-free nutrient for 2 weeks after transplanting. The leaves of the plants failed to grow and became curled and deformed. During the third and fourth weeks of treatment, pinkish brown spots appeared at the margins of the older leaves, followed by necrotic areas, a series of symptoms observed to be characteristic of severe boron deficiency in guayule. A few of the uppermost leaves expanded to some extent, but these were chlorotic and failed to attain full size as compared with similar leaves of plants grown with an adequate boron supply. In the absence of boron, the plants made no appreciable top growth and failed to flower during the first 6 weeks of treatment, while others that received boron flowered during this period. Some of the plants lacking boron were then given daily applications of nutrient containing different levels of boron in order to test their ability to recover from the deficiency. During the following 8 weeks, the average weight of plants grown without boron was only 4.2 gm., and they produced an average of one flower head per plant. Those supplied with nutrient containing less than 1.0 p.p.m. made no appreciable top growth and failed to produce an appreciable number of flower heads. Those supplied with 1.0 p.p.m. made a limited amount of growth (average fresh weight 13.7 gm.) but failed to produce many flowers. Those supplied with a solution containing 4 p.p.m. recovered to a greater extent (average fresh weight 23.9 gm., fig. 1) and produced several flower stalks per plant. The average weight of plants supplied with boron and a complete nutrient throughout the experiment was 61.6 gm., and they produced an average of 89.8 flower heads per plant.

FIG. 1.—Recovery of guayule from boron deficiency. Two plants on left grown for 14 weeks with nutrient lacking boron. Center plants grown for 6 weeks without boron, then supplied with nutrient containing 1 p.p.m. for following 8 weeks. Plants on right grown without boron for 6 weeks, then supplied with nutrient containing 4 p.p.m. for following 8 weeks.

RESPONSE TO DIFFERENT LEVELS OF BORON.—The growth of seedlings was greatly retarded in the case of all plants given boron-free nutrient during the first 6 months of the experiment. These boron-deficient plants were then supplied with nutrient which contained 0.004 p.p.m. of boron, and they made some growth; but in the following 10 months they were smaller and much more bushy than were the plants which received greater amounts (fig. 2). Their leaves were relatively small, and green instead of silvery green, as were those of

plants which received greater amounts of boron. Increasing the amount in the nutrient from 0.004 to 0.1 p.p.m. was associated with a significant increase in growth. Concentrations of boron ranging between 0.1 and 2.0 p.p.m. had no significant effect on the vegetative growth of the plants—on the basis of dry weight. The growth of plants supplied with nutrient containing more than 2.0 p.p.m. was significantly less than that of plants receiving nutrient containing 0.1–2.0 p.p.m. (table 2). Plants suffering from toxicity exhibited numerous dead leaves on the lower branches at intervals during the experiment; and they developed an open type of branching (apparently due to inhibition of lateral bud growth), in contrast to dense branching associated with boron deficiency (apparently due to inhibition of terminal but not lateral buds).

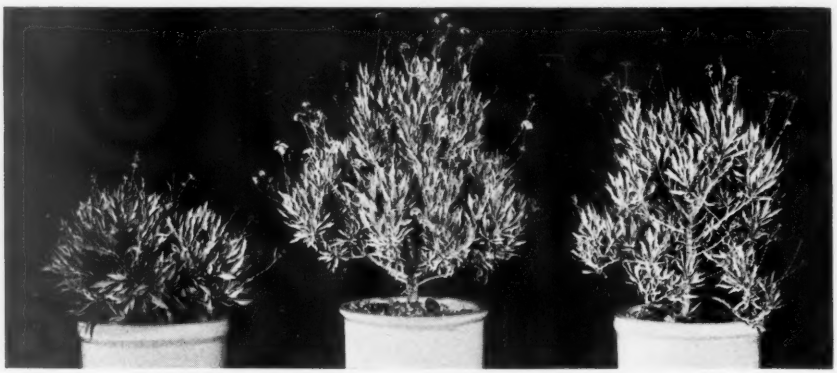

FIG. 2.—Guayule plants grown in gravel culture and supplied different amounts of boron. Left, supplied nutrient solution lacking boron for 6 months, then supplied solution containing 0.004 p.p.m. boron for following 10 months. Center, nutrient containing 0.5 p.p.m. for 16 months. Right, 10 p.p.m. of boron for 16 months.

TABLE 2

EFFECT OF NUTRIENT CONTAINING DIFFERENT LEVELS OF BORON ON PLANT WEIGHT AND ON AMOUNT OF RUBBER AND RESIN PRODUCED BY GUAYULE GROWN IN SAND CULTURE FOR 16 MONTHS

Boron in nutrient (p.p.m.)	Fresh weight per plant (gm.)	Dry weight, stems and roots (gm.)	Rubber (%)	Rubber per plant (gm.)	Resin (%)	Boron in roots and stems (p.p.m.)
0.004	203	55	3.32	1.8	3.84	18
0.014	255	69	3.86	2.7	4.07	18
0.1*	341	100	4.66	4.7	4.64	19
0.5	367	106	4.41	4.7	4.33	23
2.0	343	102	4.43	4.5	4.63	31
5.0	292	91	4.02	3.7	4.66	47
10.0	274	80	3.64	2.9	4.10	48

* For convenience, the amount of boron in tap water is not included in expressing concentrations greater than 0.014 p.p.m.

The boron content of roots and stems was relatively low as contrasted with that of seeds, particularly the seeds of plants grown with nutrient containing an ample amount (tables 1, 3). In the preliminary experiment, in which field transplants were grown in gravel culture, the leaves were also found to contain a relatively large amount of boron as contrasted with the stems, provided the plants were supplied with a nutrient containing an ample supply of boron. An increase in the amount supplied to the plants was accompanied by a corresponding increase in boron concentration in the stems, and particularly in the leaves. Slight toxicity symptoms were evident within a period of 7 weeks in plants supplied with a nutrient containing 10 p.p.m., as indicated by the presence of dead leaves, and these symptoms became more pronounced with increasing amounts of boron.

RUBBER AND RESIN.—The application of nutrient containing less than 0.1 or more than 2.0 p.p.m. of boron significantly reduced the concentration of rubber in the stems and roots and also the total rubber output of the plant (table 2). Variations in boron supply had only a very slight effect on the percentage of resin in the stems and roots.

STEM ANATOMY.—Both a deficiency and an excess of boron in the nutrient solution significantly decreased growth of the stems in diameter (table 4). Increasing the boron content of the nutrient from 0.1 to 10 p.p.m. did not significantly change the percentage of bark or wood in the cross-sectional areas. The percentage area of bark in cross-sections of stems of plants supplied with nutrient containing 0.004 p.p.m. was significantly greater than that of others supplied with larger amounts of boron. A relatively large proportion of fiber was observed in the bark, both in those plants that showed boron deficiency and in those that showed toxicity symptoms.

The cellular structure of the lower stem showed none of the irregularities in cell growth or differentiation, or the disorganization and necrosis described by others (**11, 12**) for plants grown under

TABLE 3

EFFECT OF BORON SUPPLY ON AVERAGE NUMBER, SIZE, AND BORON CONTENT OF SEEDS PRODUCED DURING DIFFERENT SEASONS OF THE YEAR BY GUAYULE PLANTS GROWN IN GRAVEL CULTURE. FIGURES REPRESENT AVERAGE NUMBER OF SEEDS PRODUCED PER PLANT DURING SUMMER (JUNE 1 TO SEPTEMBER 1) AND FALL (SEPTEMBER 1 TO OCTOBER 16), AND PERCENTAGE OF LARGE, MEDIUM, AND SMALL SEEDS

Boron in nutrient (p.p.m.)	No. of seeds produced		Percentage						Boron content, summer collection (p.p.m.)
			Small		Medium		Large		
	Summer	Fall	Summer	Fall	Summer	Fall	Summer	Fall	
0.004	1380	626	78.5	42.0	20.9	52.8	0.7	5.2	21
0.014	2350	892	49.8	39.2	48.6	56.5	1.6	4.2	27
0.1	2519	1209	18.6	13.5	64.6	70.5	16.9	16.0	30
0.5	2220	1183	20.1	14.2	62.4	68.1	17.5	17.7	43
2.0	2282	1109	15.5	14.5	59.3	66.9	25.2	18.6	71
5.0	2204	1062	17.7	16.5	53.7	65.6	28.6	18.0	82
10.0	2138	881	15.6	13.3	56.0	69.8	28.5	16.9	212

more severe stress of boron deficiency or toxicity. In plants deficient in boron (supplied 0.004 p.p.m. in the nutrient) compared with those receiving nutrient containing 0.1 p.p.m., parenchyma tissue of the bark appeared to be similar in cell size and tissue organization. The area of undifferentiated tissue adjacent to the cambium, as measured from the innermost mature phloem fiber to the outermost mature xylem, formed a slightly greater proportion of the stem tissue in plants supplied with nutrient containing 0.004 p.p.m. of boron than it did in stems of those supplied 0.1 p.p.m.

SEEDS.—Plants of a different variety (406) produced a relatively large number of seeds when supplied with nutrient containing a wide range of boron levels—from 0.004 to 5 p.p.m. (table 3). The use of nutrient containing a higher level (10 p.p.m.) did not decrease seed production appreciably, but the application of deficient amounts (0.004 p.p.m.) greatly reduced seed production. Application of nutrient deficient in boron was associated with the production of a relatively high percentage of small seeds, while the use of nutrient containing 0.1–10 p.p.m. resulted in the production of a relatively large proportion of medium and large-sized seeds.

The percentage germination of large seeds was significantly greater than that of medium or small-sized seeds (table 5), and that of seeds produced through the use of solutions containing 0.1 p.p.m. boron was significantly greater than that of seeds produced by plants receiving smaller amounts. There was no significant difference in the percentage germination of seeds obtained through the use of nutrient solutions that contained 0.1–10.0 p.p.m. The percentage germination of seeds collected during September and October was significantly greater than that of seeds collected during the summer months.

TABLE 4

EFFECT OF BORON SUPPLY ON CROSS-SECTIONAL AREAS OF STEM AND COMPONENT TISSUES. FIGURES REPRESENT AVERAGES OF EIGHT PLANTS EACH

BORON IN NUTRIENT (P.P.M.)	TOTAL STEM AREA ($MM.^2$)	WOOD		BARK		BARK FIBERS	
		Area ($mm.^2$)	Total stem area (%)	Area ($mm.^2$)	Total stem area (%)	Area ($mm.^2$)	Bark area (%)
0.004	133.2	51.87	38.8	82.04	61.3	22.99	28.5
0.1	211.6	90.74	42.9	120.77	57.1	29.06	24.3
0.5	231.6	98.24	42.5	133.12	57.5	32.34	24.6
10.0	148.2	63.76	43.2	84.55	56.9	23.82	28.7

Discussion

In nutrient cultures, the boron requirements of guayule were similar to those of some plants reported by others —such as tobacco, lettuce, soybean, cotton, broad bean, and sunflower (3, 5, 9)—in that it required approximately the same optimal concentration. Guayule apparently differs from most of these plants in that it has a slightly wider tolerance range, from 0.1 to approximately 2.0 p.p.m. MCHARGUE (5)

found the optimal range for lettuce to be 0.4–0.8 p.p.m., but 0.9 produced symptoms of toxicity. EATON (3) found that 0.5 was optimal for the sunflower and that symptoms of toxicity appeared in plants grown with a 1.0 p.p.m. boron supply. EATON also stated that 0.5 p.p.m. is sufficient for most plants, or may be excessive for some. The relatively wide boron-tolerance range of guayule may enable it to grow in some of the high-boron soils found within the cultivated guayule range, or when irrigated with water which has a relatively high boron content.

When supplied with amounts of boron outside the limits of its tolerance range (0.1–2.0 p.p.m.), the vegetative growth of gravel-culture guayule plants was reduced. Since this decrease in the dry weight of the plants was associated with decrease in the rubber content of the roots and stems, the effect on total rubber output was marked. Plants supplied with nutrient containing a deficient amount of boron (0.004 p.p.m.) produced 60% less, and those supplied with nutrient containing an excessive amount (10.0 p.p.m.) produced 35% less, rubber than did others given an optimum supply.

The growth resulting from cambial

TABLE 5

EFFECT OF BORON SUPPLY ON GERMINATION OF SEEDS FROM PLANTS GROWN IN GRAVEL CULTURES COLLECTED DURING SUMMER (JUNE 1 TO SEPTEMBER 1) AND FALL (SEPTEMBER 1 TO OCTOBER 16)

COLLECTION DATE	BORON IN NUTRIENT SOLUTION (P.P.M.)						
	0.004	0.014	0.1	0.5	2.0	5.0	10.0
	Large-sized seeds						
Summer	30.75	18.75	49.25	58.50	49.50	41.75	45.75
Fall	34.75	35.25	57.50	65.50	61.75	59.25	59.75
	Medium-sized seeds						
Summer	8.50	12.75	26.00	29.75	34.25	30.50	23.25
Fall	32.75	32.00	39.75	49.25	46.25	41.75	42.50
	Small-sized seeds						
Summer	1.00	2.25	4.50	5.75	5.75	4.75	7.25
Fall	10.00	9.75	16.25	14.75	17.00	18.50	18.25
	Mean of all sizes						
Summer	13.50	11.25	26.50	31.25	29.75	25.75	25.50
Fall	25.75	25.75	37.75	42.50	41.75	39.75	40.25

activity, as seen in the total stem area, is also unfavorably affected by either a deficiency or an excess of boron. With excess (10 p.p.m.) there was an over-all decrease in the formation of both wood and bark tissues. In the case of deficiency (0.004 p.p.m.), the significantly greater proportion of bark to wood (both composed mainly of secondary tissues) suggests that cambial activity was altered as the result of treatment. Histological symptoms, characteristic of pronounced boron toxicity or deficiency, were absent from the meristematic tissues of the lower stems.

It is reported (7) that the quantity of seeds produced by legumes was greatly reduced when the plants were grown with an inadequate boron supply but that their quality with respect to germination was not changed. Boron deficiency (0.004 p.p.m.) in guayule was associated with the production of relatively few seeds and also with a decrease in their quality, reflected in both size and percentage of germination. Relatively high concentrations of boron occurred in the seeds, as compared with the stems, and the amount of boron in the seeds varied widely, depending upon the amount available to the plant. Seeds containing less than 30 p.p.m. of boron were relatively low in percentage germination. Seeds that contained 30 p.p.m., however, were approximately the same with respect to germination as other seeds that contained 212 p.p.m. These results indicate that the quality of seeds as to size and germination produced by guayule grown on soils having a relatively high boron content would depend largely upon factors other than the available boron supply.

Summary

1. Plants of guayule were grown from early seedling stage in gravel culture for a period of 16 months, during which time they were supplied with nutrient solutions containing 0.0–10.0 p.p.m. of boron. In other experiments, somewhat older seedlings were transplanted from the field and grown in sand or gravel cultures to determine the effects of deficient or excessive amounts of boron (0.0–40.0 p.p.m.) when these were supplied by means of nutrient solutions.

2. The plants showed marked boron-deficiency symptoms when supplied with a boron-free nutrient, but they subsequently grew vigorously and produced seeds when supplied with a nutrient solution containing 4 p.p.m.

3. According to results based on vegetative growth in gravel cultures, guayule has a tolerance range of from 0.1 to 2.0 p.p.m. of nutrient solution, a range somewhat wider than that reported for most crop plants of a mesophytic type.

4. Data on percentage of rubber in gravel-culture plants show an optimum boron requirement of 0.1–2.0 p.p.m., a concentration range similar to that required for maximum vegetative growth and seed production. The concentration of rubber in the stems and roots of boron-deficient plants was lower than that of similar parts of plants grown with an adequate supply.

5. Since boron deficiency was associated with both reduced vegetative growth and lower percentage of rubber, the total rubber output of boron-deficient plants was relatively small. An excessive amount of boron (10 p.p.m. in the nutrient) was also associated with a decrease in rubber output. Over the range studied, the amount of boron supplied to the plants had only a very slight effect on the concentration of resins in their stems and roots.

6. The stem diameters of gravel-culture plants showed that cambial activity was somewhat reduced in the plants

receiving nutrient containing 10.0 p.p.m. of boron, and also in the stems of plants that received a deficient amount. The proportions of pith, xylem, bark, and bark fiber in the basal region of the stem were not altered as the result of supplying the plants with nutrient containing various levels of boron (0.01–10.0 p.p.m.). In stems of plants grown with a limited supply (0.004 p.p.m.) the area of bark was greater, in proportion to the amount of wood produced, than in comparable segments of stem from plants grown with an adequate boron supply.

7. Boron-deficient plants produced fewer seeds than did plants supplied with an adequate amount. Seeds produced by boron-deficient plants were smaller and their germination was lower than those from plants grown with an adequate supply. With respect to size, number, and percentage germination, the quality of seeds produced by plants supplied with nutrient containing an excessive amount of boron (10 p.p.m.) was equal to that produced by plants grown with a moderate supply (0.1 p.p.m.). Boron accumulated in the seeds (and also leaves) of the plants grown with an adequate boron supply.

All rubber and resin determinations were made by R. L. HOLMES, boron determinations by GLEN EDGINGTON, and statistical analyses by MARY W. SHANOR.

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THE KNOWN GEOGRAPHIC DISTRIBUTION OF THE MEMBERS OF THE VERBENACEAE AND AVICENNIACEAE. SUPPLEMENT 2

HAROLD N. MOLDENKE

Since the publication of my original compilation of the known geographic distribution of the 2966 accepted members of the families Verbenaceae and Avicenniaceae[1] and its first supplement,[2] based chiefly on an examination of 75,000 herbarium specimens in 142 private and institutional herbaria, continued collecting by numerous colleagues and correspondents and the examination of more herbarium material have brought to light 312 additional records which are presented herewith. These represent 33 new country or island records, 73 state or province records, and 206 county or parish records. Four new taxonomic entities are proposed and described.

CANADA:

Ontario

Verbena hastata L. (Algoma and York Counties)

Verbena urticifolia L. (Simcoe County)

Manitoba

Verbena bracteata Lag. & Rodr.

UNITED STATES OF AMERICA:

Maine

Verbena hastata L. (Waldo County)

New York

Verbena hastata L. (Cortland and Rensselaer Counties)

Verbena urticifolia L. (Cortland and Rensselaer Counties)

Pennsylvania

Verbena hastata L. (Bradford County)

Verbena simplex Lehm. (Centre County)

Virginia

Verbena simplex Lehm. (Henrico County)

Verbena urticifolia L. (Henrico County)

Verbena urticifolia var. *leiocarpa* Perry & Fernald (Campbell County)

West Virginia

Verbena urticifolia L. (Jackson County)

Verbena urticifolia var. *leiocarpa* Perry & Fernald (Wyoming County)

North Carolina

Verbena bonariensis L.

Verbena urticifolia var. *leiocarpa* Perry & Fernald (Catawba County)

South Carolina

Verbena tenuisecta Briq. (Lexington County)

Georgia

Callicarpa americana L. (Oglethorpe County)

Verbena simplex Lehm. (Fulton County)

Verbena tenuisecta Briq. (Ware County)

Florida

Clerodendrum bungei Steud. (Leon County)

Lantana involucrata L. (Palm Beach County)

[1] MOLDENKE, H. N., The known geographic distribution of the members of the Verbenaceae and Avicenniaceae. Pp. 104. New York City. 1942.

[2] ———, The known geographic distribution of the members of the Verbenaceae and Avicenniaceae. Supplement 1. Pp. 4. New York Botanical Garden. 1943.

Lantana montevidensis (Spreng.) Briq. (Citrus County)
Phyla nodiflora (L.) Greene (Collier and Pinellas Counties)
Verbena maritima Small (Broward County)
Verbena tenuisecta Briq. (Bay County)

Alabama
Callicarpa americana L. (Elmore County)
Clerodendrum bungei Steud. (Montgomery and Tuscaloosa Counties)
Phyla lanceolata (Michx.) Greene (Montgomery County)
Verbena tenuisecta Briq. (Butler County)
Verbena xutha Lehm. (Montgomery County)

Mississippi
Verbena rigida Spreng. (Warren County)
Verbena xutha Lehm. (Harrison, Hinds, and Warren Counties)

Ohio
Verbena simplex Lehm. (Adams and Highland Counties)
Verbena urticifolia L. (Ross County)

Illinois
Phyla lanceolata (Michx.) Greene (Alexander, Franklin, Johnson, McHenry, and Vermilion Counties)
Verbena bracteata Lag. & Rodr. (Champaign and Jackson Counties)
Verbena canadensis (L.) Britton (Union County)
×*Verbena engelmannii* Moldenke (Adams County)
Verbena hastata L. (Vermilion County)
×*Verbena rydbergii* Moldenke (Champaign County)
Verbena stricta Vent. (Union County)
Verbena urticifolia L. (Adams, Vermilion, and Woodfred Counties)
Verbena urticifolia var. *leiocarpa* Perry & Fernald (Alexander County—not "Miami County" as erroneously stated in Supplement 1)

Indiana
Phyla lanceolata (Michx.) Greene (Bartholomew, Floyd, Huntington, Jennings, Johnson, Madison, Miami, Parke, Shelby, Tipton, and Wabash Counties)
Verbena bracteata Lag. & Rodr. (Cass, Grant, Howard, Miami, Montgomery, and Starke Counties)
×*Verbena engelmannii* Moldenke (Boone, Carroll, Cass, Franklin, and Hancock Counties)
Verbena hastata L. (Bartholomew, Boone, Clinton, Dearborn, Franklin, Fulton, Gibson, Hancock, Howard, Lawrence, Madison, Miami, Randolph, Sullivan, Vigo, and Wabash Counties)
Verbena simplex Lehm. (Carroll, Kosciusko, and Lawrence Counties)
Verbena stricta Vent. (Howard, Miami, and Tipton Counties)
Verbena urticifolia L. (Boone, Clinton, Crawford, Franklin, Hamilton, Hendricks, Kosciusko, Madison, Miami, Montgomery, Ohio, Owen, Ripley, Tippecanoe, and Wabash Counties)
Verbena urticifolia var. *leiocarpa* Perry & Fernald (Brown, Decatur, Dearborn, Greene, Hamilton, Hancock, Jefferson, Miami, Monroe, Parke, and Randolph Counties)

Kentucky
Verbena hastata L. (Hopkins County)
Verbena simplex Lehm. (Larue County)

Tennessee
Verbena canadensis (L.) Britton (Wilson County)
Verbena simplex Lehm. (Wilson County)

Michigan
Verbena bracteata Lag. & Rodr. (Menominee County)
Verbena hastata L. (Mackinac and Menominee Counties)
Verbena stricta Vent. (Menominee County)
Verbena urticifolia L. (Menominee County)

Wisconsin
Verbena bracteata Lag. & Rodr. (Walworth County)
Verbena hastata L. (Vilas County)
Verbena urticifolia L. (Lafayette County)

Minnesota
Verbena bracteata Lag. & Rodr. (Rice County)
Verbena hastata L. (Rice County)
Verbena stricta Vent. (Fillmore County)
Verbena urticifolia var. *leiocarpa* Perry & Fernald (Rice County)

South Dakota
Verbena bracteata Lag. & Rodr. (Custer and Pennington Counties)
Verbena hastata L. (Pennington County)

Missouri
×*Verbena illicita* Moldenke (Cooper County)

Arkansas
Verbena bracteata Lag. & Rodr. (Garland County)
Verbena canadensis (L.) Britton (Chicot, Conway, Franklin, Pope, and Yell Counties)
Verbena halei Small (Calhoun County)
×*Verbena illicita* Moldenke (Baxter County)
Verbena litoralis H.B.K. (Bradley County)
×*Verbena moechina* Moldenke (Newton County)
Verbena stricta Vent. (Baxter, Johnson, Pope, Searcy, and Van Buren Counties)
Verbena tenuisecta Briq. (Calhoun County)
Verbena urticifolia L. (Cross, Garland, Johnson, Marion, Pope, and Yell Counties)
Verbena urticifolia var. *leiocarpa* Perry & Fernald (Newton and Phillips Counties)

Louisiana
Callicarpa americana L. (Livingston Parish)
Phyla incisa Small (Bossier Parish)
Verbena bonariensis L. (Tangipahoa and Terrebonne Parishes)
Verbena halei Small (Iberville, Saint James, and Tangipahoa Parishes)
Verbena scabra Vahl (Tangipahoa Parish)
Verbena urticifolia L. (Bossier Parish)
Verbena xutha Lehm. (Saint James Parish)

Montana
Verbena bracteata Lag. & Rodr. (Park County)

Wyoming
Verbena stricta Vent. (Weston County)

Utah
Phyla cuneifolia (Torr.) Greene (Emery County)

Nevada
Verbena bracteata Lag. & Rodr. (Elko County)

Nebraska

Phyla cuneifolia (Torr.) Greene (Hall County)
Phyla lanceolata (Michx.) Greene (Cass and Washington Counties)
Verbena bipinnatifida Nutt. (Furnas County)
Verbena bracteata Lag. & Rodr. (Washington County)
Verbena canadensis (L.) Britton (Furnas County)
Verbena hastata L. (Washington County)
Verbena stricta Vent. (Adams, Cherry, Nemaha, and Washington Counties)
Verbena urticifolia var. *leiocarpa* Perry & Fernald (Washington County)

Oklahoma

Verbena bracteata Lag. & Rodr. (Bryan County)
Verbena canadensis (L.) Britton (Bryan County)
Verbena stricta Vent. (Bryan County)
Verbena urticifolia L. (Bryan County)

Texas

Lantana horrida H.B.K. (Llano County)
Phyla incisa Small (Edwards and Presidio Counties)
Tetraclea coulteri A. Gray (Presidio County)
Verbena bipinnatifida Nutt. (Gonzales County)
Verbena canadensis (L.) Britton (Culberson County)
Verbena halei Small (Grimes County)
Verbena neomexicana var. *xylopoda* Perry (Presidio County)

Arizona

Verbena ciliata Benth. (Mohave County)

California

Phyla incisa Small (Kings County)
Phyla lanceolata (Michx.) Greene (Kings County)
Verbena gooddingii var. *nepetifolia* Tidestr. (San Bernardino County)

MEXICO:

Phyla nodiflora (L.) Greene (Michoacán)
Phyla scaberrima (A. L. Juss.) Moldenke (Chiapas)
Priva aspera H.B.K. (Tamaulipas)
Priva mexicana (L.) Pers. (Tamaulipas and Zacatecas)
Stachytarpheta mutabilis var. *violacea* Moldenke (Michoacán)
Stachytarpheta violacea Miranda (Puebla)*
Tetraclea coulteri A. Gray (Tamaulipas)
Verbena ehrenbergiana Schau. (Tamaulipas)
Verbena elegans var. *asperata* Perry (Coahuila and Zacatecas)
Verbena gracilis Desf. (Coahuila)
Verbena litoralis H.B.K. (Tamaulipas)
Verbena perennis Wooton (Tamaulipas)

COSTA RICA:

The "+" should be deleted after the name *Citharexylum macradenium* Greenm., since this species, formerly known only from Costa Rica, has now been found in Panama.

PANAMA:

Citharexylum macradenium Greenm. (Coclé)

The "+" should be deleted after the name *Citharexylum macrochlamys* Pittier, since this species, formerly known only from Panama, has now been found in Colombia.

ST. BARTHOLOMEW:

Lantana camara var. *flava* (Medic.) Moldenke
Lantana camara var. *sanguinea* (Medic.) L. H. Bailey
Phyla nodiflora (L.) Greene
Phyla nodiflora var. *reptans* (H.B.K.) Moldenke

COLOMBIA:

Aegiphila aculeifera Moldenke (Méta)
Aegiphila martinicensis Jacq. (Santander Norte)
Avicennia nitida Jacq. (Nariño)
Citharexylum macrochlamys Pittier (El Cauca)
Duranta repens L. (Nariño)
Duranta sprucei Briq. (Putumayo)
Lantana armata Schau. (Santander Norte)
Lantana boyacana Moldenke (Santander Norte)
Lantana canescens H.B.K. (Santander Norte)
Lantana canescens var. *integrifolia* Moldenke (Cundinamarca)*
Lantana glandulosissima Hayek (Santander Sur)
Lantana moritziana Otto & Dietr. (Putumayo and Santander Norte)
Lantana rugulosa H.B.K. (Putumayo)
Lippia schlimii Turcz. (Caldas and Santander Sur)
Phyla scaberrima (A. L. Juss.) Moldenke (El Valle)
Stachytarpheta cayennensis (L. C. Rich.) Vahl (Santander Norte)
Stachytarpheta cayennensis f. *albiflora* Moldenke (El Cauca)
Verbena litoralis H.B.K. (Santander Norte)

VENEZUELA:

Bouchea prismatica (L.) Kuntze (Carabobo)
Citharexylum spinosum L. (Aragua)
Lantana camara var. *mista* (L.) L. H. Bailey (Bolívar)
Lantana trifolia L. (Carabobo)
Lippia berterii Spreng. (Bolívar)
Phyla betulaefolia (H.B.K.) Greene (Carabobo)
Stachytarpheta australis Moldenke (Carabobo)
Stachytarpheta cayennensis f. *albiflora* Moldenke (Bolívar)
Stachytarpheta mutabilis (Jacq.) Vahl (Lará)
Vitex orinocensis var. *multiflora* (Miq.) Huber (Carabobo)

ECUADOR:

Duranta repens L. (Manabí)
Lantana scabiosaeflora H.B.K. (Azuay)
Verbena microphylla H.B.K. (Leon)

PERU:

Castelia cuneato-ovata Cav. (Tacna)
Duranta sprucei Briq. (Puño)
Junellia juniperina var. *grisea* (I. M. Johnst.) Moldenke (Tacna)
Verbena berterii (Meisn.) Schau. (Ayacucho and Huancavelica)
Verbena clavata Ruíz & Pav. (Tacna)
Vebena glabrata H.B.K. (Ayacucho)
Verbena variabilis Moldenke (Huancavelica)*

BRAZIL:

Aegiphila scandens Moldenke (Amazonas)

BOLIVIA:

Lantana rugulosa H.B.K. (Cochabamba)

CHILE:

Urbania egañioides R. A. Phil. (Tarapacá)

INDIA:

Clerodendrum fragrans var. *pleniflorum* Schau. (United Provinces)
Premna barbata Wall. (Punjab)

HAWAII:

Lantana camera var. *aculeata* (L.) Moldenke (Oahu)

SAMOA:

Clerodendrum inerme (L.) Gaertn. (Ofu, Tau, and Tutuila)
Clerodendrum speciosissimum Van Geert (Ofu and Tau)
Stachytarpheta urticaefolia (Salisb.) Sims (Tau)
Vitex trifolia var. *bicolor* (Willd.) Moldenke (Tau)

NIUE (SAVAGE) ISLAND:

Clerodendrum inerme (L.) Gaertn.
Clerodendrum speciosissimum Van Geert
Premna corymbosa (Burm. f.) Rottl. & Willd.
Premna tahitensis var. *rimatarensis* F. H. Br.
Stachytarpheta urticaefolia (Salisb.) Sims
Verbena officinalis L.
Vitex trifolia var. *bicolor* (Willd.) Moldenke

CULTIVATED:

Aloysia virgata (Ruíz & Pav.) A. L. Juss. (Uruguay)
Citharexylum fruticosum var. *subvillosum* Moldenke (New York)
Clerodendrum bungei Steud. (Alabama)
Clerodendrum heterophyllum (Poir.) R. Br. (Australia)
Clerodendrum speciosissimum Van Geert (Niue Island)
Clerodendrum thomsonae Balf. f. (Niue Island)
Duranta repens L. (St. Bartholomew)
Duranta serratifolia (Griseb.) Kuntze (Uruguay)
Holmskioldia sanguinea Retz. (St. Bartholomew)
Lantana camara L. (Indiana)
Lantana camara var. *mista* (L.) L. H. Bailey (Uruguay)
Lantana foetida Rusby (Uruguay)
Stachytarpheta mutabilis var. *violacea* Moldenke (California)
Tectona grandis L. f. (Niue Island)
×*Verbena hybrida* Voss (St. Bartholomew)
Verbena peruviana (L.) Britton (St. Bartholomew)
×*Verbena wingei* Moldenke (England)*
Vitex agnus-castus L. (Oklahoma)

Lantana canescens var. **integrifolia** Moldenke, var. nov.

Haec varietas a forma typica speciei foliis integris recedit.—This variety differs from the typical form of the species in having its leaf-blades entire-margined. The type was collected by H. Garcia y Barriga (*no. 10615*) at La Vega on the road to Nocaima, alt. 950–1200 m., Cundinamarca, Colombia, between June 27 and 29, 1942, and is deposited in the United States National Herbarium at Washington.

Stachytarpheta cayennensis f. **albiflora** Moldenke, f. nov.

Haec forma a forma typica speciei corollis albis recedit.—This form differs from the typical form of the species in having white corollas. The type was collected by Dr. José Cuatrecasas (*no. 14156*) on the Pacific side of the Río Micay, alt. 5–20 m., Guayabal, El Cauca, Colombia, on February 26, 1943, and is deposited in the Britton Herbarium at the New York Botanical Garden.

Verbena variabilis Moldenke, sp. nov.

Herba perennis; caulibus leviter puberulo-strigillosis stramineis; ramis gracillimis plusminus puberulento-strigillosis; foliis sessilibus oppositis vel approximatis tripartitis revolutis utrinque strigillosis; spicis dense multifloris, rhachide dense patento-puberulo.

Perennial herb 50–100 cm. tall; stems tough, subterete or slightly tetragonal, lightly puberulent-strigillose, glabrescent toward the base, stramineous; branches short, very slender, more or less puberulent-strigillose; principal internodes 3–5 cm. long; leaves sessile, decussate-opposite or approximate, rather uniformly green on both surfaces, 2–3 cm. long, 1–2 cm. wide, the lower ones and those on the stems in general 3-parted with broad segments, each segment entire or with 1–3 large lobes, those on the branches 3-parted with very narrow, linear, pinnatisect segments, the lobes blunt at apex, all strigillose on both surfaces, revolute along the margins; peduncles slender, 2.5–9.5 cm. long, more or less strigillose-puberulent, especially toward the apex, usually with 1 or 2 pairs of much reduced tripartite bracts near the apex and at the midpoint; spikes 2–5 cm. long, densely many-flowered; rachis densely spreading-puberulent; bractlets lanceolate, about 5 mm. long, long-acuminate, densely glandular-puberulent with erect spreading hairs; calyx about as long as the subtending bractlet, thin, lightly glandular-puberulent; corolla light-purple, white within, reddish-pink at base, its tube somewhat exserted from the calyx.

The type of this species was collected by R. D. Metcalf (*no. 30255*) in moist clay soil in good open drainage near Córdova, alt. 3050–3300 m., prov. Castrovirreina, Huancavelica, Peru, on March 27 or 28, 1942, and is deposited in the United States National Herbarium at Washington. The roots are used by the Indians against fleas.

×**Verbena wingei** Moldenke, nom. nov. *Verbena tenera* × *Aubletia* Winge, Proc. Linn. Soc. London **150**: 236. 1938.

NEW YORK BOTANICAL GARDEN
NEW YORK CITY, NEW YORK

FURTHER OBSERVATIONS ON THE TELEMORPHIC EFFECTS OF CERTAIN GROWTH-REGULATING SUBSTANCES[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 564

J. M. BEAL

Introduction

The formative influences induced in plants by a number of growth-regulating substances have been described by ZIMMERMAN (3, 4) and ZIMMERMAN and HITCHCOCK (5, and other papers). Some of the most active of these were certain of the substituted phenoxy compounds, application of which they found resulted in modification of the pattern of leaves, flowers, and fruits, and in changing the correlation phenomena of organs. Five of these compounds have been used in experiments described in the present paper, and five additional substances have also been tested on one of the experimental plants.

Because pronounced morphological responses are often incited or induced in plants at considerable distance from the point of application of the growth-regulating substance, the term "telemorphic effect" or response has been proposed to cover them (1). These previously described experiments have since been repeated and extended, and additional data have been obtained.

The use of Carbowax as a carrier for growth-regulating substances was recently described by MITCHELL and HAMNER (2), and in my later experiments both Carbowax 1500 and lanolin have been employed for this purpose. The results of ZIMMERMAN and of ZIMMERMAN and HITCHCOCK had shown strikingly different responses or effects induced by certain of the substituted phenoxy compounds. The experimental data presented here show that the carrier in which a specific compound may be applied may also profoundly influence the character and degree of the response. Sweet pea (*Lathyrus oderatus*), African marigold (*Tagetes erecta*), and Red Kidney bean (*Phaseolus vulgaris*) have been employed as experimental plants.

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

Observations

SWEET PEA

Since the previous report (1) describing the telemorphic effects in the sweet pea resulting from application of 4-chlorophenoxyacetic acid in lanolin, another series of plants has been grown in the greenhouse in 4-inch pots containing fertile soil. Six to eight plants were grown in each pot, and all in a given pot received the same treatment. The chlorophenoxy compounds were made up in 1% and 0.25% mixtures, in both lanolin and Carbowax 1500, and applied as small pellets near the center of the upper surface of the two leaflets of the first well-developed foliage leaf. The plants were observed daily for 2 weeks or more, or until they died—as occurred in several instances before that length of time had elapsed.

2-CHLOROPHENOXYACETIC ACID.—This substance resulted in only slight epinasty and stem curvature, whether applied in lanolin or in Carbowax. Enlargement of roots did not result, but the total root development was less than in control plants. Nearly all plants were

dead at the end of 2 weeks when Carbowax was the carrier, but when lanolin was used little effect was observed, the plants being similar to untreated controls.

4-CHLOROPHENOXYACETIC ACID.—Striking differences in responses occurred when this acid was used. When applied in Carbowax the leaves showed epinasty but the stems showed little or no curvature (fig. 1*A*). Both epinasty and stem curvature, however, quickly became evident when lanolin was the carrier (fig. 1*B*). But a more striking difference was in the effects produced on the roots of the treated plants. No visible enlargement was observed when a 1% mixture of the substance in Carbowax was used. A 0.25% mixture in Carbowax resulted in slight thickening of roots on a portion of the plants. The roots of plants to which the same substance was applied, either as a 1% or a 0.25% mixture in lanolin, were often thickened for variable distances just back of the tips. The enlarged region varied from 1 mm. to 30 mm. in length and was usually two to three times the diameter of equivalent-aged roots from control plants at 8–10 days following treatment. Sections of these roots have again shown the same histological effects as those illustrated in the former paper (1). The plants began to die at 10 days; but, unlike those treated with 2-chlorophenoxyacetic acid, death occurred earlier when lanolin was the carrier. All the plants had died at 20 days after treatment with the 1% mixtures, but most of those treated with the weaker mixtures were still alive and growing after 4 weeks, although many of them had greatly enlarged stems.

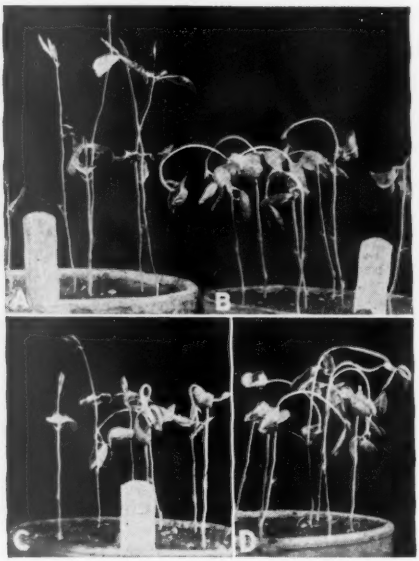

FIG. 1.—Sweet pea plants 48 hours after placing on first well-developed leaf a small pellet of: *A*, 1% mixture of 4-chlorophenoxyacetic acid in Carbowax; *B*, 1% mixture of same in lanolin; *C*, 1% 2,4-dichlorophenoxyacetic acid in Carbowax; *D*, 1% mixture of same in lanolin.

2,4-DICHLOROPHENOXYACETIC ACID.—The plants to which this acid (fig. 1*C*, *D*) was applied reacted similarly, whether Carbowax or lanolin was the carrier, so far as epinasty and stem curvature were concerned. There was a difference in the responses of roots, however. The 2,4-dichlorophenoxyacetic acid as a 1% mixture in either carrier produced only a slight effect on the roots, and one not easily found; a 0.25% mixture in Carbowax also resulted in scarcely observable enlargement; and the same concentration in lanolin was nearly as effective as the 4-chlorophenoxyacetic acid in lanolin.

2,4,5-TRICHLOROPHENOXYACETIC ACID.—A 1% mixture in Carbowax resulted in slight root enlargement only, while a 1% mixture in lanolin, as well as 0.25% mixtures in both Carbowax and lanolin, resulted in pronounced root enlargement.

2,4,6-TRICHLOROPHENOXYACETIC ACID.—A 1% mixture in lanolin resulted in scarcely visible responses on either leaves or stems, and these disappeared completely after 2–3 days. Growth then continued for a period of 2–3 weeks, when the terminal buds became yellowed, the leaf primordia thickened, and elongation ceased. The stem also thickened just back of the terminal bud, the entire plant began to lose its green color, and death followed shortly thereafter.

Each of the preceding phenoxy compounds resulted in noticeable stem swelling at various places on many of the plants, even though not applied directly to the stems. Only a few of the plants to which 2,4,5-trichlorophenoxyacetic acid was applied in lanolin developed adventitious roots, and these appeared almost entirely at and just above the ground line, never involving a length greater than 1 inch.

AFRICAN MARIGOLD

The African marigold is so sensitive and responds so readily to such a variety of stimuli that perhaps it is not a good experimental subject. Some of the results obtained in two series of experiments have been so striking, however, that a record of them seems warranted. The first series of plants was grown in the greenhouse during early spring, in flats containing fertile garden soil. When the first pair of true leaves had expanded the plants were treated by applying 1% lanolin mixtures of the compounds as a narrow ring around the first internode. Four of the chlorophenoxy compounds were used.

2-CHLOROPHENOXYACETIC ACID.—A few of the plants to which this acid was applied showed a slight swelling of the internode at the region of application after 3–4 days but practically no epinasty or stem bending. The plants seemed to recover completely, showing no observable effects after a week, and during a period of 4 weeks of observation they developed essentially as did controls.

4-CHLOROPHENOXYACETIC ACID.—The plants to which this acid was applied showed much more evident effects. Conspicuous galls, which attained a diameter two to three times that of the stem above and below the region of application, developed on all the treated plants (fig. 2*B*). There was marked suppression of root development as compared with controls (fig. 2*A*). The petioles of leaves distal to the place of application became much enlarged and yellowed and the terminal buds so distorted in form and structure after 2 weeks as to be no longer functional in further leaf production.

FIG. 2.—African marigold plants with roots in water cell: *A*, control, same age as *B*, plant at 5 weeks after ringing stem (shown by arrow) with 1% mixture of 4-chlorophenoxyacetic acid in lanolin.

2,4,5-TRICHLOROPHENOXYACETIC ACID.—Application of this acid resulted in pronounced epinasty and stem curvature within a few hours. Within 48 hours the curvature was so great that in some plants the growing points were turned

directly downward. As growth was resumed, some plants assumed an S shape (fig. 3*B*), while others formed a complete loop. The leaves of these plants yellowed in 5–7 days following treatment, and all the plants were dead within 2 weeks.

2,4,6-TRICHLOROPHENOXYACETIC ACID.—No visible response occurred on either leaf or stem for approximately 2 weeks following application. Newly developing leaves appearing after 2 weeks were strongly attenuated and mottled. The terminal buds soon became malformed and no longer functional in further leaf development, and plants started dying 3–4 weeks following treatment.

A second series of plants was grown under similar conditions a little later in the spring, and when they had attained a height of approximately 10 inches they were treated by spraying on their leaves and stems the substituted phenoxy compounds dissolved in a 5% aqueous solution of Carbowax. About thirty plants were used for each of the compounds, and the mixtures were applied in the form of a fine mist with a small hand-operated atomizer.

2-CHLOROPHENOXYACETIC ACID.—A concentration of 0.13% resulted in pronounced epinasty and stem curvature at 24 and 48 hours following treatment, but the plants then seemed to recover gradually, so that at 96 hours there was little visible effect. Newly developed leaves and their leaflets were smaller and more slender than on control plants, and their color was a paler green. Numerous small glistening protuberances became evident on the lower portions of the stems at 9–12 days, the epidermis and outer cortex split 5–7 days later, and roots emerged through the fissures. By now the terminal buds were distorted and no longer functional. Many plants were dead at 3 weeks and practically all of them were at 4 weeks following treatment.

4-CHLOROPHENOXYACETIC ACID.—A 0.06% mixture in 5% Carbowax resulted in both epinasty and stem curvature within 24 hours, followed by gradual

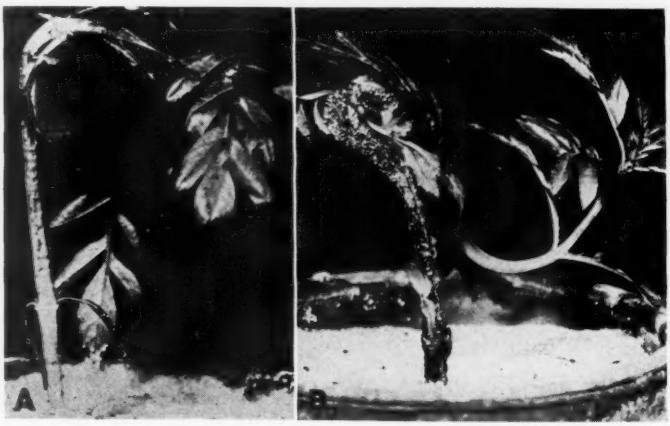

FIG. 3.—Marigold plants sprayed with: *A*, 0.06% aqueous solution of 2, 4-dichlorophenoxyacetic acid in 5% Carbowax (after 15 days); *B*, 0.06% aqueous solution of 2,4,5-trichlorophenoxyacetic acid in Carbowax (after 28 days).

recovery, however, so that 4 days after treatment no effect was apparent. The plants continued apparently normal development for at least another week, after which the newly formed leaves were somewhat attenuated. The effect was not great enough to prevent continued growth, and these plants were vigorous and nearly normal in appearance 4 weeks after treatment.

2,4-DICHLOROPHENOXYACETIC ACID.—Concentrations of both 0.06 and 0.13% resulted in marked epinasty and stem curvature within a few hours, followed by enlargement of the stems throughout most of their length after 3–4 days (fig. 3*A*). The epidermis of the swollen stems ruptured, the cortex often split longitudinally, and roots emerged—chiefly from the fissures—at about 3 weeks following treatment. The plants had now begun to lose their green color and quickly yellowed and died. Only two were living after 4 weeks, and they were dead 3 days later.

The plants reacted more quickly to the 0.13% than to the 0.06% mixture, and fewer plants produced adventitious roots, mainly because nearly half of them were dead 2 weeks after treatment. The responses were otherwise essentially alike for both concentrations.

2,4,5-TRICHLOROPHENOXYACETIC ACID.—This was applied also as 0.06% and 0.13% mixtures in Carbowax. The effects were essentially like those produced by the same concentration of 2, 4-dichlorophenoxyacetic acid. Noticeable swelling, splitting of epidermis and cortex, and emergence of adventitious roots from the enlarged stems were conspicuous features (fig. 3*B*). Death did not occur quite so quickly following treatment with this acid as with the 2, 4-dichlorophenoxyacetic acid, but only one plant remained alive at 35 days.

BEAN

Three series of experiments, starting May 9, June 7, and September 30, respectively, dealing with the effects of the same five chlorophenoxy compounds have been carried out on the Red Kidney bean. In addition, indole-3-acetic acid, α-naphthaleneacetic acid, β-naphthoxyacetic acid, tryptophan, and naphthyl acetamide were applied to some of the plants in the last series. The chlorophenoxy compounds were applied as 1% and 0.25% mixtures in both Carbowax and lanolin, while the other substances were applied only as 1% mixtures in the two carriers.

The bean plants were grown in the greenhouse in flats containing fertile loam soil. Approximately thirty seeds were planted in each flat and after germination were thinned for uniformity and desired spacing, usually to twenty plants per flat. When the heart-shaped leaves had reached nearly full size (fig. 4*B*), a small pellet of one of the mixtures was applied to the upper surface near the base of both leaf blades of all the plants in a given flat. The mixture usually was placed in direct contact with the upper surface of the pulvinus, an effort being made to apply as nearly as possible equal volumes of the mixture to each leaf and to each plant. This volume has been estimated to average approximately 40 mg. per plant and to carry either 400 γ in the 1% mixtures or 100 γ in the 0.25% mixtures. There was some variation in the amounts applied to different plants, and there were differences in the responses made by individual plants, but not all the variations resulted from this cause. It is well known that differences in responses of individual plants to almost any type of stimulus occur, but in these experiments striking differences

in responses to or in the effects resulting from the application of a given compound have also been observed to depend on the carrier used.

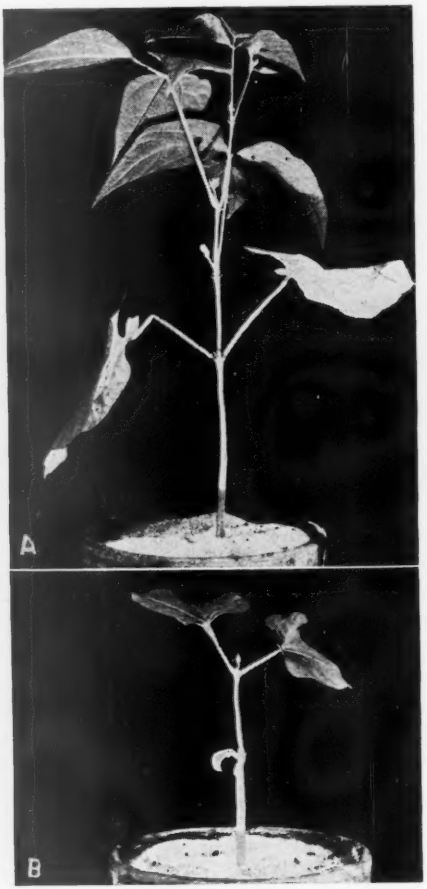

FIG. 4.—*A*, control plant of kidney bean, same age as those shown at 15 days after treatment; *B*, showing stage of development attained when mixtures were applied to bases of heart-shaped leaves.

2-CHLOROPHENOXYACETIC ACID.—A 1% mixture in Carbowax resulted in epinasty at 24 hours following application, with only about half the plants showing relatively slight stem curvature. The leaves gradually recovered, but stem curvature increased during the next 2–3 days, producing a permanent bend in the first internode of nearly half the plants. Petioles of some of the heart-shaped leaves began to thicken in diameter on the third or fourth day, and the stems of many of the plants became swollen both above and below the second node (fig. 5*A*, *B*). The swelling occurred only above this node in some plants, only below it in others, and both above and below in still others. As a consequence of the increased diameter the epidermis often split and the cortex became fissured longitudinally for varying distances. On approximately one-third of the plants the heart-shaped leaves began to yellow and die at 7–8 days after treatment. These plants were usually dead by the end of 2 weeks. The terminal buds were killed on almost all the plants at 2 weeks, as were the axillary buds at more than half the first and second internodes. At 18–20 days after treatment most of the surviving axillary buds at the second node began to develop, followed 3–4 days later by some at the first node. Their development was slow, however, and small, narrow, and usually rolled leaflets developed. Their further expansion was slow, and on none of the plants observed over a period of 5 weeks were any of them more than one-third the size of the leaflets of control plants (fig. 4*A*). Most were misshapen and mottled, and none of the plants had flowered at 4 weeks. Several produced abundant adventitious roots at 5 weeks when grown under humid conditions (fig. 6), and a few plants flowered at approximately 5 weeks after treatment and formed viable seeds.

A 0.25% mixture in Carbowax resulted in similar but much less intense effects. Elongation of the second inter-

node and expansion of the first trifoliate leaf began at about 72 hours after treatment. The leaflets were attenuated and curled, much as with the 1% mixture, but they expanded somewhat more and were a much darker green than those of controls. In a few plants the second internodes, terminal buds, and petioles of the first trifoliate leaves showed light yellow glistening swellings after 7 days. 72 hours. The rate and character of growth seemed unaffected (fig. 5*A*, *C*), and blooming occurred at the same time as in controls. A 0.25% mixture in lanolin resulted in no visible effects. All plants treated with this acid in lanolin set pods as abundantly and as early as the controls.

4-CHLOROPHENOXYACETIC ACID.—A 1% mixture in Carbowax resulted in no

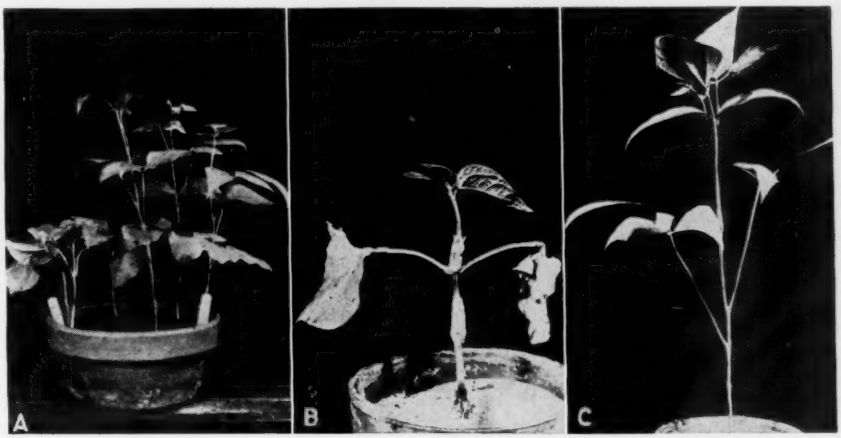

FIG. 5.—Effects of 1% mixture of 2-chlorophenoxyacetic acid on growth of bean: *A*, applied in Carbowax to three plants at left and in lanolin to three at right (after 12 days); *B*, effect in individual plant from Carbowax mixture after 21 days; *C*, same from lanolin mixture after same interval.

The second and third trifoliate leaves, evident at 14–17 days, showed even more distorted leaflets (fig. 12*E*). Axillary buds at the first and second internodes started expanding at 3 weeks, and their leaflets were also distorted. Flowers opened on four plants 20 days after treatment, but no pods had set 10 days later. None of the plants was killed by this treatment (fig. 6).

In striking contrast, the application of a 1% mixture in lanolin resulted in practically no visible effects. Slight epinasty was detectable at 24 hours, but this had disappeared completely before visible effects for a period of approximately 48 hours. A few plants showed epinasty and slight stem curvature at 72 hours, but there was no apparent retardation of growth. The first trifoliate leaves came at the same time as on controls and appeared normal in all respects. Nearly half the second trifoliate leaves were definitely mottled and darker green than those of controls. All the third and subsequent leaves were also mottled (fig. 7*A*, *B*) but were dwarfed little or none. Flowering was not retarded nor was the setting of pods or seed development interfered with.

Plants treated with a 0.25% mixture in Carbowax showed slight epinasty at 24 hours, but this disappeared before 72 hours had elapsed. No stem curvature and no retardation of growth occurred.

FIG. 6.—Effects of 0.25% mixture of 2-chlorophenoxyacetic acid in Carbowax 40 days after treatment. Both terminal and axillary buds killed; enlargement of hypocotyl and development of adventitious roots through essentially the entire length.

These plants developed apparently normally, except that they might possibly have grown slightly taller than the controls, but this may have been caused by some other factor or factors of the environment.

When lanolin was used as the carrier the responses were quicker and decidedly greater in degree than when Carbowax was used, in marked contrast to the effects of 2-chlorophenoxyacetic acid in the same carriers.

A 1% mixture of 4-chlorophenoxyacetic acid in lanolin resulted in both epinasty and stem curvature 24 hours following application, the degree increasing gradually up to 72 hours. Terminal bud expansion was slowed little or none, but the leaflets of the first trifoliate leaf were markedly attenuated and rolled upward. The internodes appeared somewhat more elongated than those of controls, as did also the petioles of the trifoliate leaves. Only one plant showed any swelling of the terminal bud. The rolling and attenuation of the leaflets were conspicuous at 7–8 days following treatment, and the unrolling and expansion were slow. As the leaflets unrolled and expanded on the tenth to fourteenth days (fig. 7*A*, *C*), they were strongly mottled and much darker green than those of controls. Blooming was not delayed, but nearly all flowers were distorted and no pods had set 10 days after the first flowers had opened. Later-formed leaves generally remained small and narrow (fig. 12*G*).

The effects of a 0.25% mixture in lanolin were almost as marked as those resulting from the 1% mixture of the same acid. Growth of the terminal bud began as early as on untreated plants, but the leaflets of the first trifoliate leaf were strongly attenuated and curled. After 4–6 days they had expanded somewhat but were crinkled and mottled, with dark green splotches. Leaves developed from axillary buds were similar, and the whole aspect of these plants was like those treated with 0.25% 2-chlorophenoxyacetic acid in Carbowax. A typical leaf at 28 days is shown in figure 12*H*. The plants flowered at the same time as controls, but the flowers were malformed

and no pods had set at 35 days following treatment.

2,4-DICHLOROPHENOXYACETIC ACID.—Application of a 1% mixture in Carbowax resulted in epinasty and stem curvature within 6 hours following treatment. At 24 hours the curvature was so great as to invert the growing points and heart-shaped leaves of nearly all plants. The petioles of a few leaves were beginning to swell, and after 48 hours petioles second node and terminal bud, in some on the hypocotyl and terminal bud, in others on the first and second nodes and terminal buds, and in still others it was essentially throughout the plants. Nearly all terminal buds became so swollen and distorted as to be nonfunctional, and hence there was little growth of trifoliate leaves (fig. 8*C*). Half of all treated plants died within 2 weeks following treatment, but a few of the survivors began to re-

FIG. 7.—Effects of 1% mixture of 4-chlorophenoxyacetic acid on growth of bean: *A*, applied in Carbowax to four plants at left and in lanolin to three at right (after 12 days); *B*, effect on individual plant from Carbowax mixture after 21 days; *C*, same from lanolin mixture after same interval.

and second internodes of most plants were distinctly enlarged. Epinasty and stem curvature remained essentially unchanged during the following week. In the meantime, enlargements of the hypocotyls (principally near the ground line but at places above also) of the first internodes and of petioles continued. The heart-shaped leaves on about half the plants began to yellow, and six of eighteen plants had died 9 days after treatment. There was no uniformity among the plants as to the place or places at which telemorphic effects occurred; in some plants the enlargement was on the cover. Three had functional terminal buds in the last series, and these began growing after about 16 days. The leaves produced were attenuated and strongly mottled, but one plant flowered on the twenty-fifth day after treatment. Pods were setting on the three plants at 35 days (fig. 8*E*).

A 0.25% mixture in Carbowax resulted in epinasty and stem curvature at 24 hours which persisted for 3–4 days. The terminal buds developed almost as quickly as those of controls but produced attenuated and mottled leaflets on only a part of the plants (fig. 8*A*). The leaflets

of the first trifoliate leaf were not rolled but were much darker green than those of the controls. The second and subsequent trifoliate leaflets were still more attenuated, slightly rolled, and mottled (fig. 12*D*). Stem enlargement occurred in only a few plants and there only below the cotyledonary node. On the whole these plants responded similarly, but somewhat less, than those treated with 0.25% 2-chlorophenoxyacetic acid in Carbowax or with 1% 4-chlorophenoxyacetic acid in lanolin. Blooming was not delayed, and fruit setting occurred—but at a reduced rate.

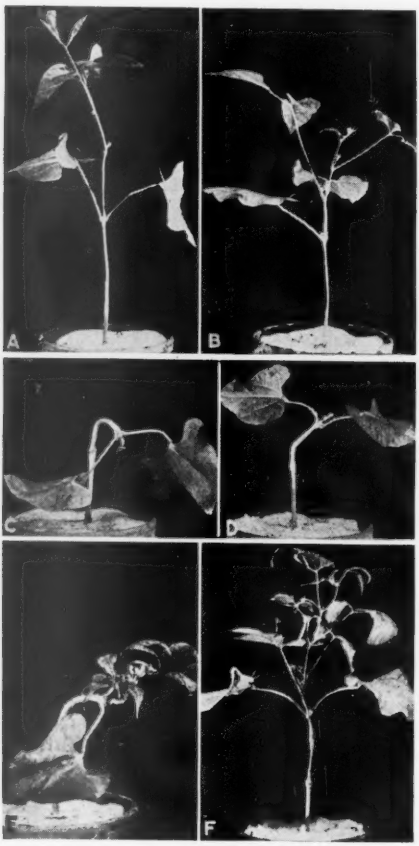

FIG. 8.—Effects of 2,4-dichlorophenoxyacetic acid on bean plants applied as: *A*, 0.25% mixture in Carbowax; *B*, same in lanolin (both after 15 days); *C*, 1% mixture in Carbowax; *D*, same in lanolin (both after 15 days); *E*, *F*, relative stages of recovery from 1% mixtures of this acid in Carbowax and lanolin, respectively, at 35 days following treatment.

A 1% mixture applied in lanolin resulted in the same general effects as when applied in Carbowax. The swelling of hypocotyls, first and second internodes, petioles, and terminal buds paralleled closely in the two carriers, but with the telemorphic effects rather less severe when lanolin was the carrier (fig. 8*D*). The leaf bases and pulvini where the mixture was applied showed considerable enlargement at 8–9 days, with some of the heart-shaped leaves beginning to yellow. Two of twenty treated plants were dead after 2 weeks, and only three after 35 days. About half the surviving plants started recovering after 16–17 days, but their progress was slow (fig. 8*F*). The new leaves were small, slightly rolled, and generally mottled. Flowering was delayed for more than a week as compared with controls, but pods set abundantly.

A 0.25% mixture in lanolin had essentially the same effects as the same concentration in Carbowax (fig. 8*B*). In both instances the first trifoliate leaves were scarcely affected, while the second and later ones were dwarfed and mottled, with much darker green areas (fig. 12*C*).

2,4,5-TRICHLOROPHENOXYACETIC ACID.—A 1% mixture in Carbowax resulted in telemorphic effects strikingly similar to those resulting from application of a 1% mixture of 2,4-dichlorophenoxyacetic acid in the same carrier. Epinasty, stem curvature, stem and petiole swelling, retardation of normal growth, yellowing of heart-shaped leaves,

and death of treated plants were at essentially the same rate and degree from both substances. Only two plants remained alive 30 days after treatment, and they were decidedly yellowed and possessed no living buds. Neither trifoliate leaves nor flowers were developed (fig. 9*A*).

A 0.25% mixture in Carbowax resulted in epinasty but no stem curvature. Terminal bud growth started almost as soon as in controls, but the rate of expansion of the trifoliate leaves was much slower. Total swelling of stems and petioles was much less than in the 1% Carbowax mixture, although 6 days after treatment the hypocotyls on the majority of the plants showed enlargement at and above the ground line, with development of adventitious roots in this region. A small percentage of the plants showed enlargement of the hypocotyl, up to and including the cotyledonary node. Root primordia were generally evident at points along the swellings (fig. 9*C*). The base of the heart-shaped leaves, together with the pulvinus and sometimes the adjacent portion of the petiole, where the mixture was applied, were often yellowed and greatly enlarged. Many of these leaves gradually lost their chlorophyll and died at 18–21 days. Terminal bud growth was much retarded, but the trifoliate leaves showed very little modification in form. A slight mottling was observed on the first-formed, but subsequent ones were nearly normal in appearance. Blooming was delayed a few days, but fruit setting was not apparently affected.

A 1% mixture in lanolin produced effects scarcely distinguishable from those just described, unless perhaps swelling of stems and slowing of growth were more pronounced (fig. 9*B*). None of the plants died from the treatment, but most first trifoliate leaflets were distorted and greener than controls. The later-formed leaves were normal in appearance.

A 0.25% mixture in lanolin produced effects similar to those described in both preceding lots. Growth was retarded at about the same rate, swellings as to position and degree were similar, and the trifoliate leaves were mottled to about the same extent (fig. 9*D*). Subsequent development was similar in the three groups.

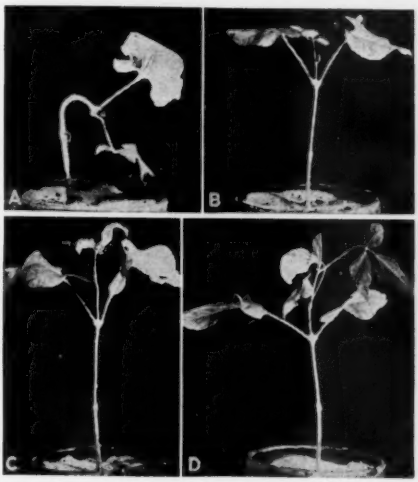

FIG. 9.—Effects of 2,4,5-trichlorophenoxyacetic acid on bean plants applied as: *A*, 1% mixture in Carbowax; *B*, 1% mixture in lanolin; *C*, 0.25% mixture in Carbowax; *D*, 0.25% mixture in lanolin. All at 15 days following application.

2,4,6-TRICHLOROPHENOXYACETIC ACID.—A 1% mixture of this substance in Carbowax resulted in neither epinasty nor stem curvature. Development of the terminal buds was a little slower than on controls, and the first trifoliate leaf showed darker green and mottled leaflets at 6 days. Subsequent trifoliate leaves were smaller than the first and more strongly mottled, with darker green

areas, and were somewhat crinkled after 15 days (fig. 10*A*). Stems showed no effects, nor was blooming delayed. The plants set fruits as freely and as early as the controls, in spite of the mottled and smaller foliage leaves (fig. 12*F*).

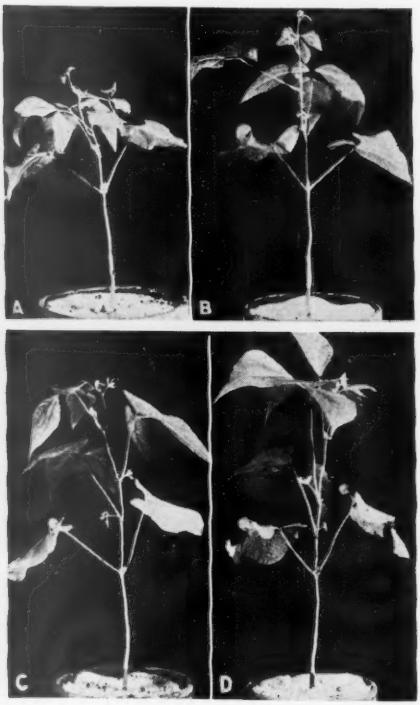

FIG. 10.—Effects of 2,4,6-trichlorophenoxyacetic acid on bean plants applied as: *A*, 1% mixture in Carbowax; *B*, 1% mixture in lanolin; *C*, 0.25% mixture in Carbowax; *D*, 0.25% mixture in lanolin. All at 15 days following treatment.

Plants treated with a 0.25% mixture in Carbowax showed no observable effects within 9–10 days, except that an occasional first trifoliate leaf showed a very slight amount of mottling. The second and subsequent trifoliate leaves were smaller, definitely mottled, and darker green than the first (fig. 10*C*), although leaves on one or two of the twenty plants were apparently unaffected. Blooming and fruit setting were not delayed.

A 1% mixture in lanolin had no apparent effect during the first week following application. When the second trifoliate leaves began to expand, some of them showed slight mottling but no other effect (fig. 10*B*). Their subsequent growth and development appeared normal.

A 0.25% lanolin mixture apparently resulted in no visible effect on the plants to which it was applied. They developed in all respects as did the controls (fig. 10*D*).

TRYPTOPHAN, INDOLE-3-ACETIC ACID, β-NAPHTHOXYACETIC ACID, α-NAPHTHALENEACETIC ACID, AND NAPHTHYL ACETAMIDE.—Each of these substances was applied as a 1% mixture in both Carbowax and lanolin to the heart-shaped leaves of approximately twenty bean plants in the manner previously described for the phenoxy compounds. Except for moderate epinasty induced by the application of indoleacetic and naphthaleneacetic acids, none of the others—except the naphthoxyacetic acid—resulted in any apparent responses during the 5-week period the plants were under observation.

β-naphthoxyacetic acid resulted in slight epinasty and scarcely detectable mottling of the trifoliate leaves when lanolin was the carrier (fig. 12*A*). The leaflets were slightly greener than those of controls, but there was neither stem swelling nor retardation of growth or blooming (fig. 11*B*). The slight mottling disappeared, and the leaves quickly became normal in size and form.

When Carbowax was the carrier the degree of epinasty was essentially the same, but the amount of mottling of the trifoliate leaves was strikingly increased

(fig. 11*A*), giving the effect of a virus disease with small dark green areas inclosed by the pale yellow translucent veinlets (fig. 12*B*).

Discussion

Since MITCHELL and HAMNER have called attention to Carbowax as a carrier of growth-regulating substances and as a means of increasing their effectiveness as compared with water solutions,

FIG. 11.—Effects of 1% mixtures of β-naphthoxyacetic acid on beans applied in: *A*, Carbowax, 22 days after application; *B*, lanolin, 28 days after application.

it seemed desirable to compare Carbowax with lanolin in similar tests.

From the detailed experiments carried out it is obvious that the effectiveness of a carrier depends upon the growth substance employed. For example, 2-chlorophenoxyacetic acid resulted in striking effects on the bean when applied either as a 1% or a 0.25% mixture in Carbowax, while the same concentrations in lanolin induced comparatively slight responses. In the case of the sweet pea the results were similar. Also, when 2,4,5-trichlorophenoxyacetic acid was dispersed as a 1% mixture in Carbowax such profound responses were induced in beans that all plants died in 3–4 weeks, while the same concentration in lanolin showed its chief effects in the slowing of growth. A 1% mixture of naphthoxyacetic acid in Carbowax resulted in a conspicuous mottling of the first and second trifoliate leaves of the bean, and usually of the third; the fourth and later

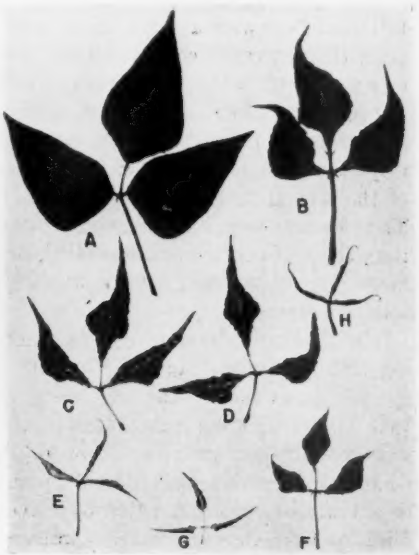

FIG. 12.—Trifoliate leaves of bean illustrating effects resulting from application of: *A*, 1% mixture of naphthoxyacetic acid in lanolin; *B*, 1% mixture of same in Carbowax; *C*, 0.25% mixture of 2,4-dichlorophenoxyacetic acid in lanolin; *D*, 0.25% mixture of same in Carbowax; *E*, 0.25% mixture of 2-chlorophenoxyacetic acid in Carbowax; *F*, 1% mixture of 2,4,6-trichlorophenoxyacetic acid in Carbowax; *G*, 1% mixture of 4-chlorophenoxyacetic acid in lanolin; *H*, 0.25% mixture of 4-chlorophenoxyacetic acid in lanolin. All 28 days after treatment—except *B*, which is 13 days.

ones were generally unaffected. The same concentration in lanolin resulted in no apparent effect on any of the leaves. On the other hand, when 2,4-dichlorophenoxyacetic acid was applied either to bean or to sweet pea, the responses were essentially the same whether Carbowax or lanolin was used as the carrier.

A striking difference in the responses occurred from the application of 4-chlorophenoxyacetic acid. When lanolin was used as the carrier with bean plants the responses were conspicuous. The growth rate was slowed; the trifoliate leaves were attenuated and dwarfed and curled upward, and never expanded to full size. They were a much darker green color than leaves of controls. Both flowering and fruit setting were delayed. On the contrary, when this acid was applied in Carbowax practically no observable responses occurred until the appearance of the second or third trifoliate leaves. Their leaflets were darker green in color than those of controls, and mottled, but flowering was not delayed nor was fruit setting reduced.

The strikingly different effects resulting from the application to plants of a specific growth-regulating substance, as well as from similar substances in the two carriers, are puzzling. How or in what form the substance enters the plant is not known. Does it enter by direct diffusion from the carrier or in combination with the carrier, as a solute in water associated with the carrier, as an ion of the acid, or does the substance become changed into another compound which is responsible for the reactions of the plant? Until answers can be supplied, at least to some of these questions, it seems futile to indulge in much speculation. That these and similar experiments with growth-regulating substances are significant is beyond doubt, however, because of the possibility of establishing for them economic and practical applications as growth stimulators, growth depressors, or herbicides.

Summary

1. Five substituted phenoxy compounds have been applied to the sweet pea, African marigold, and Red Kidney bean in both Carbowax 1500 and lanolin as carriers. In addition, indole-3-acetic acid, α-naphthaleneacetic acid, β-naphthoxyacetic acid, tryptophan, and naphthyl acetamide were applied to the bean in both carriers.

2. The data indicate that the effectiveness of the carrier depends upon the growth-regulating substance employed. In these experiments, greater growth responses and form changes resulted, on the whole, when Carbowax was the carrier, although with some substances the responses were essentially equal with both carriers, and with one substance (4-chlorophenoxyacetic acid) the responses were much greater when lanolin was the carrier.

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CHLORINE ACCUMULATION IN DATE PALM VARIETIES

A. R. C. HAAS

Introduction

Among the many varieties of date palms grown in the Coachella Valley in southern California, the Deglet Noor is commercially the most important. Although certain diseases (**1**, **2**) somewhat restrict its widespread use in some gardens, nevertheless this variety, because of the desirable qualities of its fruit, is being planted extensively.

Fruit quality may be the result of many factors, some of which do not lend themselves readily to exact measurement. An analysis of any differences in the chemical composition in the vegetative and reproductive phases of growth in the various varieties is therefore desirable, since chemical composition forms an excellent basis for estimating fruit quality.

This paper compares the total chlorine content of the pinnae and fruit from a great number of date varieties, including Deglet Noor. Samples were collected in several widely separated gardens, covering a considerable range of chloride concentration in the soil. Many of the varieties were also available in close proximity to one another in an individual garden in which the soil conditions for each variety were comparable. A comparison is also made of the chlorine content in date fruits (monocots) with that in citrus fruits (dicots)—both from orchards in low and in high chlorine soil areas.

Date palms are obtained as rooted offshoots from the parent palm, and hence the top and root are of the same variety. The tree is considered to be resistant to alkali salts (**6**), although it makes its best growth in the Coachella Valley in well-drained soils supplied with adequate quantities of irrigation water of good quality. Because of its alkali tolerance, it is generally assumed that a certain amount of salt may be beneficial in the growth of the date palm, although data in this regard are still lacking.

The pinnae are highly siliceous, containing 9–25% of silica in the dry matter (**2**). The ash ranges from 12 to 29%, about 80% of which is silica (**2**).

Within the Deglet Noor variety, a remarkable uniformity was found (**5**) in the chemical composition of the fruit samples obtained from fertilizer plots. The determinations included: total soluble solids, insoluble residues, sugar, soluble solids not sugars, and total nitrogen (including nitrate nitrogen). The fertilizer appeared to influence only the average yield per palm.

It is known that the fruits of different varieties may differ in the type of sugar contained in the pulp; some—such as Halawy and Sayer—contain the invert type, whereas the Deglet Noor variety contains cane sugar (**7**).

The inorganic composition of date fruits (**3**) shows that the percentage of ash in the dry matter of the pulp of Deglet Noor is relatively low when compared with that of many of the other varieties. Because of the low ash values, it could not be determined whether chlorine also was low, since losses of chlorine are likely to occur in ashing unless special precautions are taken (**4**). The present results clarify the relation of chlorine with respect to the different date varieties.

Methods

The lowermost (oldest) leaf in which the pinnae were still in a healthy condi-

tion was selected. The pinnae were removed and wiped free of sand and dust. After drying in a ventilated oven at 65° C., they were finely ground in a Wiley mill and reheated at 65° C. just prior to weighing out the samples.

Large samples of several hundred mature fruits were collected except as noted.[1] The fruits were wiped free of dust after the removal of the calyx. The seed was then discarded, and the pulp finely cut to facilitate drying and placed in shallow glass dishes in a well-ventilated oven at 65° C. When dry, the pulp was allowed to cool and then finely ground in an electric coffee grinder, stored in tightly sealed glass jars, and reheated at 65° C. prior to weighing out the samples. If the dried pulp stood too long before grinding, the product became sticky and was difficult to handle; if too warm at the time of grinding the product resembled taffy. When properly cooled, the dried pulp was brittle and was easily ground.

All samples of pinnae or fruit pulp were weighed out in duplicate. Usually 50 gm. of finely ground dried pinnae or 40 gm. of ground dried fruit pulp was used for each chlorine determination.

Fifty ml. of a 5% solution of sodium carbonate was added to each porcelain dish (Champion sillimanite) containing the respective samples. Sufficient distilled water was added to wet the material thoroughly. The dishes were placed on a hot plate, and when warm the contents of each dish were stirred with a glass rod which was rinsed with distilled water and removed. This was followed by oven-drying until the material was ready for ignition. Care was exercised in the ignition, since too high temperatures without sufficient sodium carbonate present is usually accompanied by losses of chlorine (4).

Fischer burners with closed vents were used for the ignitions. When thoroughly charred and cooled, the content of a dish was broken up with hot distilled water and filtered. Several further additions of hot distilled water were made, followed by filtration. The filter paper was added to the contents of the dish; and when dry on the hot plate, the ignition was repeated as previously described. Such ignitions at low heat were greatly aided by the washing-out of the soluble salts. After two or three ignitions, 10 ml. of 1:1 nitric acid was added to each dish, and both dish and filter paper were thoroughly washed with hot distilled water. The total filtrate (usually about 400 ml.) was placed in a beaker on a hot plate, an excess of silver nitrate (chlorine-free) gradually added with stirring, and when coagulated, the precipitate was filtered off and the washings with hot distilled water continued until the filtrate was free of silver. The filter paper and its contents were dried, the dry precipitate transferred to a glazed paper, and the filter paper ignited above the crucible, after which the precipitate was added. A few drops of HCl and of HNO_3 were then added. The final heating of the crucibles was carried on at a very low heat until the precipitate just began to melt. After cooling in a desiccator, the crucibles were weighed, the silver chloride determined, and the chlorine calculated. Closely agreeing duplicate tests were conducted for each sample of pinnae or fruit pulp.

Results

As shown in table 1, three gardens afforded most of the experimental ma-

[1] Assistance in securing leaf and fruit samples was generously given by Dr. D. E. Bliss, Mr. M. M. Winslow, Mr. R. W. Nixon, and those in charge in the various gardens.

TABLE 1

CHLORINE IN DRY MATTER OF PINNAE OF DATE PALMS

Garden	Variety	Palm	Date of sampling	Total chlorine in dry matter (%)
Jennings	Deglet Noor	R8E, P10	Dec. 27, 1929	0.130
Postlethwaite	Deglet Noor	R2, P2	Nov. 27, 1929	0.167
Faries	Deglet Noor	R7E, P6S	Dec. 27, 1929	0.219
Haywood	Deglet Noor	No. 19	Dec. 14, 1928	0.226
Anderson	Deglet Noor	.....	Dec. 13, 1929	0.364
Hayes	Deglet Noor	22	Dec. 14, 1928	0.374
Russel	Deglet Noor	6/25 −100−20	Nov. 8, 1930	0.212
	Deglet Noor	.....	" " "	0.224
	Barhee	14/25 −86−22	" " "	0.309
	Kustawy	21/25 −67−14	" " "	0.319
	Halawy	22/25 −85x−46	" " "	0.347
	Zahidi	21/25 −65−4	" " "	0.377
	Deglet Beida	5/25 −30−1	" " "	0.395
	Sayer	22/25 −88−44	" " "	0.431
	Zahidi	21/25 −65−4	" " "	0.436
	Khadrawy	21/25 −67−3	" " "	0.534
U.S.D.A.	Deglet Noor	Blk. 2, R8, P7	Oct. 6, 1930	0.104
	Maktoom	Blk. 2, R7, P8	" " "	0.111
	Halawy	Blk. 1, R2, P7	" " "	0.113
	Kustawy	Blk. 2, R1, P4	" " "	0.162
	Khadrawy	Blk. 2, R1, P8	" " "	0.202
	Zahidi	Blk. 2, R2, P5	" " "	0.202
	Hayany	Blk. 1, R1, P7	" " "	0.224
	Thoory	Blk. 2, R12, P7	" " "	0.261
	Barhee	Blk. 1, R2, P5	" " "	0.287
	Saidy	Blk. 1, R3, P7	" " "	0.373
	Iteema	Blk. 1, R10, P7	" " "	0.532
Russel	Deglet Noor	R99, P5	Dec. 30, 1943	0.275
	Khadrawy	R100, P4	" " "	0.319
Mecca	Maktoom	R15, P12	Dec. 30, 1943	0.130
	Deglet Noor	R12, P4	" " "	0.152
	Hellali	R14, P12	" " "	0.247
	Dayri	R15, P6	" " "	0.260
	Barhee	R10, P12	" " "	0.262
	Halawy	R14, P4	" " "	0.298
	Zahidi	R9, P5	" " "	0.299
	Rhars	R12, P12	" " "	0.304
	Saidy	R10, P5	" " "	0.313
	Hayany	R13, P12	" " "	0.313
	Khalasa	R14, P11	" " "	0.331
	Medjool	R10, P11	" " "	0.335
	Iteema	R16, P11	" " "	0.337
	Khadrawy	R13, P4	" " "	0.386
	Kustawy	R16, P10	" " "	0.422
	Tazizoot	R16, P5	" " "	0.475
	Sayer	R15, P4	" " "	0.543
	Thoory	R11, P5	" " "	0.647

TABLE 2

CHLORINE IN DRY MATTER OF PULP (INCLUDING SKIN) OF DATE FRUITS

Garden	Sample no.	Variety	Palm	Date of sampling	Total chlorine in dry matter (%)	Total chlorine in average fruit (gm.)
Faries	39	Deglet Noor	R26, P14	Sept. 28, 1932	0.309	0.0216
Haywood	38	Deglet Noor		Sept. 28, 1932	0.173	0.0129
Haywood	105	Deglet Noor		Nov. 6, 1930	0.282	0.0227
Rosenberger	34	Deglet Noor			0.195*	0.0105*
Downing	45	Deglet Noor	R4, P9	Sept. 28, 1932	0.300*	0.0222*
Postlethwaite	46	Deglet Noor	R3, P4	Sept. 27, 1932	0.235*	0.0102*
Middleton	50	Deglet Noor	R1, P16	Sept. 27, 1932	0.375*	0.0196*
Postlethwaite	20	Deglet Noor		Sept. 24, 1930	0.535*	0.0256*
Gilette and Rosenberger	31	Deglet Noor		Oct. 6, 1930	0.450*	0.0343*
Postlethwaite	40	Menakher	R1, P2	Sept. 27, 1932	0.208	0.0211
Downing	37	Iteema	R2, P27	Sept. 28, 1932	0.352	0.0192
Downing	49	Tazizoot	R7, P27	Sept. 28, 1932	0.436	0.0258
Rosenberger	16	Saidy		Sept. 19, 1932	0.284	0.0210
U.S.D.A. at Indio		Deglet Noor (5)†		Sept. 30, 1943	0.158	0.0135
	72	Maktoom	R7, P8	Oct. 13, 1930	0.162	0.0124
		Deglet Noor (5)		Sept. 30, 1943	0.175	0.0137
	47	Deglet Noor	R8, P7	Oct. 6, 1930	0.248	0.0134
	38	Saidy	R3, P7	Oct. 6, 1930	0.262	0.0183
	37	Kustawy	R1, P4	Oct. 6, 1930	0.276	0.0125
	57	Thoory	R12, P7	Oct. 6, 1930	0.325	0.0178
	44	Zahidi	R2, P5	Oct. 6, 1930	0.352	0.0215
	41	Hayany	R1, P7	Oct. 6, 1930	0.443	0.0323
	50	Khadrawy	R1, P8	Oct. 6, 1930	0.476	0.0231
Russel Bros. tropical garden at Thermal	34	Deglet Noor	R77, P17	Sept. 20, 1932	0.218	0.0124
	28	Deglet Noor	R78, P2	Sept. 20, 1932	0.240	0.0172
	120	Barhee		Oct. 17, 1930	0.249	0.0182
	43	Deglet Noor	R78, P1	Sept. 28, 1932	0.290	0.0228
	25	Zahidi	R76, P6	Sept. 20, 1932	0.307	0.0182
	147	Kustawy		Oct. 17, 1930	0.314	0.0104
	19	Tafazwin	R77, P2	Sept. 20, 1932	0.319	0.0229
	129	Zahidi		Oct. 17, 1930	0.453	0.0265
	22	Halawy	R81, P2	Sept. 20, 1932	0.465	0.0194
	135	Khadrawy		Oct. 17, 1930	0.478	0.0269
	138	Halawy		Oct. 17, 1930	0.478	0.0237
	132	Deglet Beida		Oct. 17, 1930	0.562	0.0251
	141	Fard		Oct. 17, 1930	0.621	0.0379
	126	Ista Amran (Sayer)	22—88—44 / 25	Oct. 17, 1930	0.694	0.0482
Mecca experimental plots		Deglet Noor (6)	R12, P1	Sept. 29, 1943	0.227	0.0161
		Saidy (7)		Oct. 13, 1943	0.333	0.0290
		Halawy (8)	R14, P2	Sept. 1, 1943	0.337	0.0160
		Zahidi (6)	R9, P1	Sept. 1, 1943	0.351	0.0152
		Maktoom (10)		Oct. 13, 1943	0.378	0.0204
		Barhee (10)		Oct. 13, 1943	0.378	0.0226
		Deglet Noor (10)		Sept. 29, 1943	0.386	0.0241
		Khalasa (10)		Oct. 13, 1943	0.397	0.0227
		Deglet Noor (5)		Sept. 29, 1943	0.398	0.0243
		Thoory (8)	R11, P1	Sept. 1, 1943	0.422	0.0193
		Thoory (10)		Oct. 13, 1943	0.462	0.0191
		Dayri (8)	R15, P6	Sept. 29, 1943	0.470	0.0252
		Zahidi (9)		Oct. 13, 1943	0.525	0.0290
		Sayer (9)	R15, P1	Sept. 1, 1943	0.536	0.0207
		Saidy (7)	R10, P1	Sept. 29, 1943	0.557‡	0.0249‡
		Khadrawy (8)	R13, P1	Sept. 29, 1943	0.569	0.0330
		Tazizoot (8)	R16, P4	Sept. 1, 1943	0.618	0.0326
		Kustawy (6)	R16, P6	Sept. 1, 1943	0.628	0.0184
		Tazizoot (8)		Sept. 29, 1943	0.677	0.0402

* Decline-diseased. † Nos. in parentheses indicate numbers of fruits. ‡ Immature fruit.

terial. Samples of soil[2] at the 6–12-inch depth were taken in the U.S.D.A. garden, in which a chlorine content of 22 p.p.m. of dry soil was found. At the Mecca plots the excessive chlorine in the soil was greatly reduced prior to planting of the date offshoots through the use of sulphur followed by leaching. At the 12-inch depth the soil in the Mecca plot recently showed only 40 p.p.m. chlorine. At this same depth in the omphalia root-rot area (1) in the Russel garden, the soil contained 758 p.p.m. of chlorine, and it was here that collection of material from many palm varieties was made.

Deglet Noor pinnae vary considerably in their chlorine content, depending on the garden from which they are collected (table 1). This probably takes into account the chlorine status of the soil and irrigation water, as well as fertilization and other factors. The table indicates that, in the three principal locations in which many varieties were closely associated, the chlorine content in the dry matter in the pinnae of Deglet Noor is usually lower than in comparable samples from other varieties.

In table 2 the chlorine content is expressed as percentage of dry matter in the fruit pulp (including the skin). Variations occur in the percentage of chlorine in the pulp of Deglet Noor fruits collected in various gardens. Increased chlorine content in decline-diseased fruits possibly is related to the low dry matter content of such material.

In the dry matter of the pulp, Deglet Noor generally shows less chlorine than in comparable portions of fruits of other varieties grown in the same garden.

Since complete records were kept of the number of fruits collected and their

TABLE 3

CHLORINE IN PEEL AND PULP OF CITRUS FRUITS

FRUIT	LOCATION	DATE	NO. OF FRUITS	CHLORINE IN DRY MATTER (%)	
				Peel	Pulp
	Low chlorine areas				
Valencia orange	Citrus Exp. Sta., Riverside (pathology plots)	July 11, 1942	16	0.039	0.044
Valencia orange		Aug. 12, 1942	14	.033	.045
Valencia orange		Oct. 9, 1942	14	.014	.027
Lemon (tree-ripe)		June 19, 1942	14	0.014	0.015
	High chlorine areas				
Valencia orange	Oxnard	June 19, 1942	8	0.016	0.025
Lemon (tree-ripe)		June 19, 1942	5	.029	.025
Valencia orange	La Habra	May 3, 1943	7	.025	.039
Valencia orange		May 3, 1943	8	.031	.040
Valencia orange		May 3, 1943	11	.....	.036
Lemon (tree-ripe)		May 3, 1943	11	0.036	0.023

[2] Assistance in obtaining these soil samples and in their analyses was kindly given by Dr. D. E. BLISS and Mr. B. M. LAURANCE.

fresh and dry weights, it was possible to calculate the total chlorine in an average fruit. The size of the fruit should also have been taken into account, but these data were not available. Even without this latter factor, the data in table 2 indicate the low chlorine content per fruit in Deglet Noor, especially in the data for the U.S.D.A. garden. The data for the Deglet Noor fruits from various gardens suggest a fairly wide variation in the chlorine content in the dry matter of the pulp within the same variety in various gardens.

The percentage of chlorine in the dry matter of the pulp of date fruits, although very low in all varieties, is nevertheless high when compared with that in citrus (table 3), for in date fruits (monocots) the percentage may be ten times that found in citrus fruits (dicots).

Summary

1. The chlorine content in the dry matter in the pinnae of the Deglet Noor variety is usually lower than in comparable samples in other date varieties.

2. Variations occur in the percentage of chlorine found in the pulp of Deglet Noor fruits collected in various gardens.

3. When collected in the same garden, the dry matter of the pulp of the Deglet Noor variety generally contains a lower percentage of chlorine than is found in comparable portions of fruits of other varieties.

4. Although the chlorine content in the dry matter of date pulp may appear to be small, it is about ten times that found in citrus fruits in orchards in high chlorine areas.

CITRUS EXPERIMENT STATION
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TOXIC SUBSTANCES FROM THE CULTURE MEDIA OF GUAYULE WHICH MAY INHIBIT GROWTH

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Introduction

The question of whether or not plants may emanate substances which accumulate in the soil and which may be toxic to and inhibit growth of the same or of other species is at present a controversial one. Early in the present century, PICKERING (8) suggested that an inhibitory effect of grass on the growth of apple trees, observed by him, might be due to toxic materials derived from the grass. This work was continued (9), and the results seemed to show that in numerous combinations one species might deleteriously affect the growth of a second. More concrete evidence bearing on the possibility of toxic substances in the soil was shown by the work of SCHREINER and co-workers (12–18). Four compounds—picolinic acid, dihydroxystearic acid, salicylaldehyde, and vanillin—isolated from soils were found to be very toxic to wheat seedlings. The infertility of certain soils appeared to be correlated with the presence of one or more of these compounds. Most soil infertility, however, is now known to be associated with conditions other than the accumulation of one of these four substances.

Injurious effects of black walnut trees on other plants have been reported (6, 11), and DAVIS (4) has sought to identify the toxic principle with juglone (5-hydroxy 4 α-naphthoquinone), which is found in the black walnut tree itself. Although juglone was shown to be toxic to alfalfa and to tomato, there was no evidence that this substance is actually released into the soil, or that it is in fact a toxic agent under natural conditions. PROEBSTING and GILMORE (10) have investigated the difficulty of re-establishing peach trees in old peach orchards. After demonstrating that this phenomenon was due neither to nutrient deficiencies nor to pathogenic organisms, they found that incorporation of peach roots or alcohol extracts of peach roots in soil resulted in the appearance of toxicity symptoms in young trees planted in such soil. BENEDICT (2) also showed that dried roots of bromegrass are inhibitory to the growth of the same species and suggested that its living roots may also excrete substances toxic to the plant. BENEDICT suggests that the thinning out of bromegrass stands may be the result of the accumulation of a toxic substance from the roots. The data of MYERS and ANDERSON (7) support the contention of BENEDICT.

In the experiments on growth inhibition by dead roots or root extracts, evidence is lacking that the toxic substance is in fact given off by the roots of the living plant, or is in fact the agent responsible for growth-inhibition responses in the soil or other substratum. It might also be objected that substances extracted from dead root material are not necessarily present in the living plant. In the work reported here these objections have been met to some extent. Organic substances inhibitory to the growth of plants of guayule were obtained from nutrient solution recirculated through cultures of healthy growing guayule plants. Inhibitory substances of similar chemical properties were isolated in pure form and chemically iden-

tified from water in which guayule roots had been allowed to stand briefly. The experiments were undertaken in February, 1941.

MATERIAL AND METHODS.—Guayule plants of the strain no. 593 were used throughout. Seeds were supplied by the American Rubber Producers during 1941, and thereafter by the Special Guayule Research Project, U.S. Bureau of Plant Industry, Soils, and Agricultural Engineering. The seeds were sown in washed river sand and germinated in the greenhouse at 70°–80° F. The seedlings were transplanted to 4-mesh gravel contained either in 2-gallon glazed crocks or in 1- or 2-gallon cans which had been previously painted with a nontoxic asphaltum paint. In some cases nursery plants supplied by the Guayule Project were used. Plants were supplied daily (in some cases three times a week) with Hoagland's nutrient solution. This solution has previously been shown (3) to be optimal or near optimal for the growth of guayule. In none of the experiments concerning inhibition carried out over a period of $3\frac{1}{2}$ years did any recognizable symptoms of mineral deficiency occur.

Experimentation

LEACHING.—That substances or conditions unfavorable to the growth of guayule accumulate in containers in which the plants are actively growing is shown by the following experiment. Small nursery plants were planted in 4-mesh gravel contained in 1-gallon cans which were provided with abundant drainage and placed on a well-drained, well-lighted greenhouse bench. After 1 week, new growth began to appear on the (topped) plants, and the experiment proper was begun. Eighty containers of plants selected for uniformity were disposed in rows of four along a greenhouse bench. The plants of alternate rows served as controls and were supplied three times per week with Hoagland's solution, 250 ml. per container per application. On the remaining days of the week the containers were flushed with water. The experimental rows were supplied with nutrient solution and with water which had been leached through 4-mesh gravel in which other guayule plants were grown. To this end, vigorous fast-growing plants in 2-gallon cans were used. Three times each week nutrient solution was poured through the containers of each plant until 1 liter of leached nutrient had been collected from each. The nutrient so collected was found by analysis to have undergone no significant reduction in either nitrate or phosphate concentration. Leached nutrient was dispensed to the experimental plants at the rate of 250 ml. per container three times per week. The water with which the experimental plants were supplied was similarly leached. The containers of the donor plants (from which the leachings were obtained) were periodically flushed with water, which contained only negligible amounts of salts.

After 6 weeks of growth, those plants which received leached water and nutrient were visibly smaller than the controls. Measurements of total heights of the plants at this time are given in table 1. Two months after initiation of the experiment, all the plants were measured, harvested, dried, and weighed. Table 1 gives the data on the total dry weight per plant found in the two treatments. Plants which received leached water and nutrient were significantly retarded, both as to growth in height and as to dry-weight accumulation, in comparison with plants which received fresh water and fresh nutrient. The retarded plants of this experiment were otherwise

healthy in appearance and differed from the controls in no marked way other than in size.

This type of experiment was repeated seven times. In six cases results similar to those of table 1 were obtained; in the seventh case no retardation of growth was obtained. The cause of this discrepancy may lie in the fact that the experiplanted to river sand contained in 4-inch clay pots. All plants were given nutrient on alternate days. On the intervening days the controls were watered with tap water, while the experimental plants were watered with tap water which had been leached through gravel containing actively growing guayule plants. Conversely, leachings from tomato plants

TABLE 1

GROWTH OF GUAYULE PLANTS IN GRAVEL CULTURE AND SUPPLIED EITHER WITH NUTRIENT AND WATER OR WITH NUTRIENT AND WATER PREVIOUSLY LEACHED THROUGH OTHER GUAYULE GRAVEL CULTURES. HEIGHT MEASUREMENTS AFTER 6 WEEKS; DRY-WEIGHT MEASUREMENTS AFTER 2 MONTHS. EACH FIGURE IS MEAN OF FOUR PLANTS

Solution	Plot number										Av.
	1	2	3	4	5	6	7	8	9	10	
	Height per plant (cm.)										
Control (not leached nutrient, etc.)	22.7	21.3	21.3	21.8	22.0	21.5	21.8	21.0	21.0	22.0	21.6±0.17
Leached nutrient	16.2	19.0	15.2	18.0	15.2	14.0	13.2	15.2	16.8	18.2	16.1±0.60*
	Dry weight per plant (gm.)										
Control (not leached nutrient, etc.)	4.70	4.45	4.15	4.65	4.48	4.27	3.68	4.65	4.42	4.48	4.39±0.096
Leached nutrient	2.05	2.73	1.90	2.20	2.12	2.18	1.77	2.18	1.90	2.65	2.17±0.098*

* Difference between this value and that of control significant at 1% level.

ment in question was carried out during the winter months, when growth-inhibition effects were found to be in general less than in the spring and summer. Results generally similar to those of table 1 were also obtained when only the water and not the nutrient solution was leached.

A retarding effect of guayule root leachings was not found in either of two experiments in which this material was applied to tomato plants. In the experiments of table 2, tomato plants 3 weeks old from the time of planting were transdid not appear to retard the growth of guayule (table 3). In these experiments, vigorous gravel-grown tomato plants approximately 3 feet tall were leached as described and the leached solution applied to young guayule plants. The data of tables 2 and 3 suggest that in the retarding effect of guayule root leachings on the growth of guayule plants there may be a degree of specificity.

RECIRCULATION.—In this type of experiment nutrient solution was allowed to drip, at the rate of about 500 cc. per

hour, on crocks containing gravel cultures of guayule plants 6–12 months old. The nutrient as it drained from each crock was led into a second crock containing similar gravel and three guayule seedlings. The nutrient flowing from the plants, which in the case of the experiment of table 4 were 8 months old. Both lots of nutrient were adjusted at weekly intervals so that the concentration remained relatively constant. Table 4 gives data from an experiment of this

TABLE 2

GROWTH OF TOMATO PLANTS IN SAND CULTURE AND SUPPLIED EITHER WITH WATER OR WITH WATER LEACHED THROUGH GRAVEL CONTAINING ACTIVELY GROWING GUAYULE PLANTS. EXPERIMENT G-9 CONTINUED FOR 2 WEEKS; EXPERIMENT G-5 FOR 4 WEEKS

SOLUTION	EXPERIMENT G-9		EXPERIMENT G-5	
	No. of plants	Dry weight per plant (gm.)	No. of plants	Dry weight per plant (gm.)
Control (water not leached)	27	0.790±0.029	10	1.19±0.064
Water previously leached through cultures of guayule	18	0.787±0.028	10	1.03±0.061

TABLE 3

GROWTH OF GUAYULE PLANTS IN GRAVEL CULTURE AND SUPPLIED EITHER WITH WATER OR WITH WATER LEACHED THROUGH GRAVEL OR THROUGH GRAVEL CONTAINING ACTIVELY GROWING TOMATO PLANT. MEASUREMENTS OF HEIGHT AFTER 3 MONTHS. NONLEACHED NUTRIENT APPLIED THROUGHOUT

SOLUTION	EXPERIMENT G-17		EXPERIMENT G-18	
	No. of plants	Height (cm.)	No. of plants	Height (cm.)
Control (water not leached)	16	11.31±0.89	16	8.25±0.55
Water previously leached through 4-mesh gravel	16	10.32±0.63	16	10.00±0.47
Water previously leached through gravel culture of tomato plants	16	11.78±0.99	16	8.74±0.92

crock of seedlings was collected and recirculated through the system. Control seedlings were grown in gravel in crocks provided with a nutrient-recirculating system in which no older plants were included. Thus the control plants were merely grown in recirculated nutrient, while the experimental plants were grown in nutrient recirculated through both the seedling cultures and the older type. The seedlings whose nutrient was recirculated over older plants appeared inhibited in growth as compared with those whose nutrient was recirculated over only the seedlings themselves. No deficiency symptoms appeared in either group throughout the experiment, and the plants of the two groups appeared to differ in no marked way other than in size. The results of the experiment of

table 4 were confirmed in a second experiment. In two further tests no retardation of growth appeared in the experimental plants. These discrepant results were correlated with the fact that the large plants used in the recirculation systems of the latter two experiments did not grow actively and later were found to be severely pot-bound.

GROWTH OF SEEDLINGS UNDER OLDER PLANTS.—Guayule seedlings were planted under older, established guayule plants growing in crocks filled with 4-mesh gravel. The lower branches of the large plants were trimmed away to avoid excessive shading of the seedlings. Water and nutrient was supplied several times a day in order to minimize water and nutrient competition between the older plant and the seedling. Controls were planted in similar gravel not containing older plants. In three experiments of this kind it was found that seedlings 1.5 cm. tall, when planted under older plants, either died or were greatly retarded in growth in comparison with similar seedlings not so planted. Interpretation of this result was made uncertain, however, because of the shading and possible competitive effects due to the older plants. The experiment was therefore modified as follows. A glass container of about 500-cc. capacity and provided with drainage at the bottom was sunk in the gravel which surrounded a 5–9-months old plant cultured in a 2-gallon crock. The small container was then filled with fresh gravel. Four seedlings were planted under each large plant, two being in the glass jar. Both sets of seedlings were therefore shaded by the larger plant, but two seedlings of each lot of four were subject in addition to any influences exerted on their growth by the culture medium of the older plant. Table 5 gives the results of two experiments of this kind. In both cases, seedlings planted in jars under the older plant made more growth than the seedlings planted directly in the gravel surrounding the older plants. These two experiments indicate again that some unfavorable substance may emanate from an older guayule plant.

ASSAY FOR TOXIC ACTIVITY.—Various methods of assaying the toxic effect of solutions leached through guayule cultures were attempted. The most convenient and useful method was based on the fact that guayule seedlings make a rapid and luxuriant growth in unaerated solution culture. Seedlings 3–6 weeks old were transplanted individually to shell vials of 20-cc. capacity. Wooden boxes were constructed to hold the vials in such a way that each vial fitted closely into a hole in the top of the box, with only the apical 2–3 mm. of the vial protruding. In this way the roots of the plants were kept in darkness. Twenty vials were contained in each box. The plants were held in the vials in slits made in cork stoppers, a small piece of cotton holding each plant firmly in place. Each cork had in addition a small central hole for supplying distilled water to replace that lost in transpiration. Initial measurements of height were made on each

TABLE 4

GROWTH OF GUAYULE SEEDLINGS AFFECTED BY RECIRCULATION OF NUTRIENT SOLUTION; EXPERIMENT G-37. MEASUREMENTS AFTER 3 MONTHS. EIGHTEEN PLANTS PER TREATMENT

Nutrient recirculated	Height (cm./plant)	Dry weight (gm./plant)
Over seedlings only...	24.3±0.70	3.45
Over both seedlings and 8-months-old plants.....	17.1±1.76*	1.29

* Difference between this value and that of control significant at 1% level.

plant, and dry weights were obtained by sampling each lot. The seedlings were then allowed to grow in the greenhouse at a constant temperature of 80° F. for 2 weeks, at which time final heights and dry weights were determined. During the 2 weeks, the test plants grew an average of 3–10 mm. in height, depending on the nature of the initial planting material. Dry weight increased during the same time from an initial 7–10 mg. per plant to a final 20–30 mg. per plant.

was used as a substrate for the seedling plants unless otherwise noted. This concentration gave faster growth than lower concentrations and was somewhat superior to full strength. Although the pH of the nutrient had no influence over the range pH 5.0–7.0, all solutions were nevertheless adjusted to pH 5.5 before use. Fractions to be tested for toxic activity were always tested in a series of concentrations, each concentration being three times less than the preceding.

TABLE 5

EFFECT OF 5- OR 9-MONTHS-OLD GUAYULE PLANTS ON GROWTH OF SEEDLING GUAYULE PLANTS IN SAME CROCK. IN EXPERIMENT G-36 THE LARGE PLANTS WERE 5 MONTHS OLD; IN EXPERIMENT G-40, 9 MONTHS OLD

SEEDLING PLANTS	EXPERIMENT G-36				EXPERIMENT G-40		
	No. of plants	No. surviving	Height after 5 weeks (cm.)	Dry weight (gm./plant)	No. of plants	No. surviving	Height after 6 weeks (cm.)
In gravel contained in separate jars under large plants	8	7	6.5±0.86	0.28 ±0.044	40	26	5.15±0.376
In gravel under large plants.....	8	7	3.1±0.44*	0.084±0.0058*	40	7	2.15±0.266*

* Difference between this value and that of control significant at 1% level.

Table 6 gives data on the growth (in height and dry weight) of replicate lots of guayule plants. It may be seen that for growth in height the means from groups of ten plants vary on the average approximately 7% from the over-all mean. The data indicate that a difference of 30% or more in height between two groups of ten plants should be significant at the 5% level, while a difference of 41% or more should be significant at the 1% level. Quantitatively similar results were obtained in two other homogeneity tests. In general, differences in height growth of 50% or more may be safely taken as significant in the present assay. Hoagland's solution, diluted four times, Each concentration was in turn tested on ten plants, and for every three sets of ten test plants one set of ten controls was included. As many as 550 plants were used simultaneously in one experiment. In all, more than seventy separate experiments have been carried out and approximately 1000 fractions assayed for toxic activity.

TOXICITY OF RECIRCULATED NUTRIENT SOLUTION.—In order to obtain large amounts of leached nutrient solution, an apparatus was set up in which 5 gallons of one-half strength Hoagland's solution was distributed so that it dripped through twenty 2-gallon crocks, each containing a guayule plant 6–8 months

old and 20 cm. or more tall. The nutrient solution was collected from each crock and recirculated twice each day. Facilities for the simultaneous recirculation of 60 gallons of nutrient solution were arranged. In general, the recirculation was continued for approximately 30 days. If during the 30 days any of the plants assumed an unhealthy appearance, it was immediately replaced. Every precaution was also taken to avoid the presence of algae in the crocks and other parts of the recirculation system. This system occupied one entire greenhouse and necessitated a great deal of care.

In table 7 the results of the application of six such recirculated nutrient solutions to guayule seedlings are given. In these experiments, one-half strength Hoagland's solution was used for the control plants to simplify comparison with those grown in the recirculated one-half strength nutrient. Plants grown in recirculated nutrient were inhibited in growth as compared with control plants in fresh nutrient. In the mean, the recirculated nutrient supported only about 50% as much growth as the fresh nutrient. The seedling plants grown in the recirculated nutrient received adequate nitrogen and phosphorus from this source, as shown by analysis, and demonstrated no visible deficiency symptoms. That the relatively poor growth supported by the recirculated nutrient is actually due to a toxic substance or substances will be demonstrated in the next section.

TABLE 6

GROWTH (MM./PLANT) IN HEIGHT AND DRY WEIGHT IN REPLICATE LOTS OF SEEDLING GUAYULE PLANTS IN NON-AERATED SOLUTION CULTURE. TWO-WEEK GROWTH PERIOD. INITIAL DRY WEIGHT, 70 MG./10 PLANTS

Plant	Plot number									
	1	2	3	4	5	6	7*	8*	9*	10
1	15	8	9	7	8	8	11	8	7	3
2	4	6	5	10	5	8	6	6	5	3
3	8	8	6	7	5	9	5	4	8	7
4	7	6	5	9	10	10	7	7	4	8
5	8	6	9	15	6	9	9	10	14	5
6	3	6	7	11	6	8	8	11	6	9
7	8	7	6	12	10	5	4	7	10	9
8	7	9	11	8	11	9	11	8	8	10
9	7	5	5	5	7	6	9	8	9	6
10	6	10	2	10	9	10	8	7	9	11
Average	7.3± 1.01	7.1± 0.50	6.5± 0.82	9.4± 0.91	7.7± 0.70	8.2± 0.51	7.8± 0.74	7.6± 0.62	8.0± 0.89	7.1± 0.89
Final dry weight (mg. per 10 plants)	240	230	200	270	190	220	210	220	220	220
Increase in dry weight per 10 plants	170	160	130	200	120	150	140	150	150	150

* Received nutrient containing inactive fraction.

TOXIC SUBSTANCES IN RECIRCULATED NUTRIENT.[1]—Preliminary experiments showed that recirculated solution could be concentrated to dryness without loss of its toxicity for guayule seedlings. Ashing the dried material resulted in

[1] Preliminary work on this subject was carried out in co-operation with Dr. SIDNEY GOTTLIEB.

total loss of the toxic activity, however, indicating that the toxic activity might be due to organic material present in the recirculated nutrient. Attempts were made to extract this toxic material from the dried recirculate with organic solvents. The activity could be removed with acetone, methyl or ethyl alcohol, or by ether after acidification of the dry material. Extraction with ether from nontivity passed into the alkali, leaving an inactive resinous residue. Addition of acid to the alkali extract separated the toxic activity into two fractions, one insoluble in acid, which precipitated out, and one which remained in solution. At most, 75 mg. of alkali-soluble acid-precipitable material (fraction 4a of table 9) could be obtained from the 30-day recirculate of 240 plants. This scar-

TABLE 7

GROWTH OF GUAYULE SEEDLINGS IN NUTRIENT SOLUTION PREVIOUSLY LEACHED THROUGH CULTURES OF GUAYULE PLANTS. EACH SOLUTION ASSAYED IN FIVE SEPARATE EXPERIMENTS AND EACH FIGURE IS MEAN DERIVED FROM FIFTY PLANTS

LEACHED NUTRIENT NO.	GROWTH (MM./10 PLANTS/10 DAYS) IN:				
	Leached nutrient		Leached nutrient diluted with fresh nutrient		Control (fresh nutrient)
	Millimeters	Percentage control	Three times	Nine times	
1.....	12.6	33	22.2	24.0	37.8
2.....	23.3	50	42.0	41.8	47.0
3.....	23.3	59	34.2	36.0	39.6
4.....	18.7	71	24.6	20.6	26.2
5.....	17.8	65	25.4	23.0	27.4
6.....	18.2	66	26.4	24.8	27.4
Average..	19.0±1.63*	57.3±5.68	29.1±3.06	28.4±3.46	34.2±3.48

* Difference between this value and that of control significant at 1% level.

acidified material was much less effective in removing the toxic activity. These facts, which indicate that the toxic principle or principles may be acidic in nature, are brought out by data of typical experiments presented in table 8. Accordingly, attempts were made to enrich the toxic activity through a series of fractionations, and the result of a typical procedure is shown in table 9. By exhaustive extraction of the acidified dried recirculate with ether, most of the toxicity could be removed. The ether extract was then taken to dryness and the residue extracted with 2% NaOH. The acacity of material made it impossible to carry the fractionation substantially further than indicated in the table. From fraction 4b of table 9, 84 mg. of an unknown compound were isolated in crystalline form, m.p. 159°–161.5° C. Since this compound proved to be nontoxic, it was not investigated further. Fraction 4b also contained inactive metallo-organic compounds in considerable quantity.

About 1.8 gm. of ether-soluble material could be extracted from nutrient solution which had been recirculated over 240 guayule plants for 30 days

TABLE 8

EFFECT OF TREATMENTS ON TOXICITY TOWARD GUAYULE SEEDLINGS OF NUTRIENT RECIRCULATED OVER GUAYULE ROOTS. EACH FIGURE MEAN FROM TEN PLANTS. ALL FIGURES EXPRESSED IN PERCENTAGE OF CONTROL GROWN IN FRESH NUTRIENT. EACH FRACTION TESTED IN ALIQUOT EQUAL TO ORIGINAL FOR THAT EXPERIMENT

Fraction	Growth in height (full strength)	Percentage control	
		Diluted three times	Diluted nine times
A: 1. Original recirculate	30%*	35	75
2. 1 concentrated down and again diluted	34*	72	77
3. Distillate from concentration	96	96	112
B: 1. Original recirculate	51*	66	74
2. 1 after ashing	98	104	102
C: 1. Original recirculate	51*	53	72
2. 96% EtOH extract	58*	69	98
3. Acid-ether extract	40*	63	85
4. Residue after 3	94	87	98
5. Neutral-ether extract	78	96	83
6. Petroleum-ether extract	110	106	88

* Fraction considered active.

TABLE 9

PROCEDURE IN FRACTIONATION OF TOXIC ACTIVITY FROM NUTRIENT SOLUTION PREVIOUSLY RECIRCULATED FOR 30 DAYS OVER GUAYULE CULTURES

Fraction	Dry weight (gm.)	Activity
1. 60 gallons recirculate, concentrated to dryness		
a. Dry residue	60	Active
b. Distillate	.....	Inactive
2. 1a mixed with asbestos, acidified to pH 2.0, and extracted with peroxide-free ether		
a. Ether-soluble	1.81	Active
b. Ether-insoluble	58	Inactive
3. 2a reduced to small volume and extracted with successive portions of 2% NaOH		
a. Alkali-soluble	1.64	Active
b. Alkali-insoluble	0.16	Inactive
4. 3a acidified to pH 2.0 and precipitate filtered off		
a. Precipitated by acid	0.08	Very active
b. Not precipitated by acid (contains inorganic salts)	1.49	Active

(table 9). This amounts to approximately 7.5 mg. per plant. In one experiment the volatile material in the recirculate was caught in a CO_2 trap during the concentration, and the distillate was exhaustively extracted with ether. This yielded a total of 0.7 gm. of volatile oils, or about 3 mg. per plant. Apparently, considerable quantities of organic matter accumulate in nutrient solution which is recirculated through guayule cultures. This organic matter includes, as just shown, fractions inhibitory to the growth of guayule seedlings.

TABLE 10

INHIBITION OF GROWTH OF GUAYULE SEEDLINGS BY ADDITION OF MATERIAL OBTAINED FROM WATER IN WHICH GUAYULE ROOTS WERE ALLOWED TO SOAK FOR VARIOUS PERIODS

Exp. no.	Days soaked	Highest concentration of dried material (mg./cc.)	Growth in height (% control)		
			Highest concentration	Diluted three times	Diluted nine times
1.....	1	1.6	55	59	74
2.....	3	1.4	57	79	98
3.....	5	2.1	63	75	100
4.....	6	1.0	41	100	73
5.....	11	1.0	53	62	80

STEEPING OF ROOTS.—Since isolation of the active compound from guayule recirculate was impeded by the small amounts of original material that could be obtained, even by relatively vast amounts of labor, various other methods of obtaining larger amounts were investigated. Of these the following was most successful. Roots of guayule nursery plants (0.25–0.50 inches, caliper at crown) were packed into 2-gallon jars and the jars filled with distilled water. After 1–11 days the water was drained off, concentrated, and the residue tested for toxic activity on guayule seedlings. This material proved to be highly toxic (table 10), concentrations of 1–2 mg. per cubic centimeter giving roughly 50% inhibition of seedling growth. Steeping of the roots for more than 1 day did not appear greatly to increase the toxic activity of the solution. Solutions allowed to steep for as long as 11 days were still clear and relatively free of bacteria and possessed toxic activity toward guayule seedlings. The plants subjected to steeping also appeared healthy and could be readily transplanted after 11 days of treatment.

ISOLATION OF TOXIC COMPOUNDS FROM STEEPATE.—Table 11 gives the steps used in developing large quantities of material from steeping guayule roots. The weights and activities presented are those from two duplicate isolations. In each case approximately 20,000 nursery plants were used. These were topped at the crown, rinsed thoroughly in water, and packed tightly in a vertical position into 2-gallon glazed crocks, about 500 plants per crock. The roots were then covered with distilled water. After 4–6 days the water was drained off and the 65 gallons of light brown liquor filtered through cheesecloth, after which it was concentrated to dryness *in vacuo*. The dried material was acidified to pH 4.0 with H_2SO_4, transferred to a Soxhlet thimble, and extracted with peroxide-free ether for 32 hours. At the end of this time the mass was again acidified to pH 2.0 and extracted for an additional 24 hours. The ether extract was more toxic toward guayule seedlings than the original material, while the ether-insoluble residue was less toxic. The combined ether extracts were next concentrated to a small volume and extracted with ten to thirteen successive 20-cc. portions of 0.5 normal NaOH. This step did not

result in any marked enrichment of the activity but freed the ether extract of materials which would interfere with the next procedure. The alkali-soluble material was now acidified to pH 1.2 with concentrated H_2SO_4. After 5 days at 2° C. a copious crystalline precipitate appeared which was centrifuged off. This material (4b) was purified by sublimation, resublimation, three recrystallizations from petroleum ether, and four recrystallizations from water:

M.p.	Equivalent wt.	%C[2]	%H
84°–86° C.	174	71.7	7.3

[2] Microanalyses by Dr. G. OPPENHEIMER and Mr. G. SWINEHART.

The acid-soluble fraction 4a was adjusted to pH 4 and after 2 days at 2° C. yielded 0.23 gm. of a crystalline material (5a).

TABLE 11

ISOLATION OF TOXIC COMPOUNDS FROM GUAYULE-ROOT STEEPATE. ISOLATION NOS. 14 AND 15

Fraction	Weight (gm.)	Concentration for 50% inhibition (mg./cc.)
1. Dried material given off to distilled water by 20,000 guayule roots	460	1.5
2. Extraction of 1 (above) with redistilled ether		
a. Ether-soluble	16.9	0.5
b. Ether-insoluble	443	>3
3. Ether-soluble fraction 2a extracted with 0.5 normal NaOH		
a. Alkali-soluble	16.4	0.5
b. Alkali-insoluble	0.5	0.5
4. Fraction 3a acidified to pH 1.2		
a. Acid-soluble	16.0	0.4
b. Acid-insoluble (from isolation no. 15)	0.72	0.036
5. Fraction 4a allowed to remain at 0° in water at pH 4.0 for 2 days		
a. Precipitate (crystalline)	0.23	0.15
b. Filtrate	15.8	0.5
6. Fraction 4a distilled at atm. pressure		
a. Boiling at 63°–80° C.	0.90	>2
b. Boiling at 80–99	0.40	>2
c. Boiling at 100–109	1.03	>2
d. Boiling at 110–119	1.46	>2
e. Boiling at 120–135	1.66	0.5
f. Boiling at 136–150	2.07	0.5
g. Residue	2.38	0.5
7. Fraction 6g taken up in water and extracted with benzene		
a. Benzene-soluble (partly crystalline) (from no. 15)	1.51	0.1
b. Benzene-insoluble (from no. 15)	0.47	>3
8. Fraction 4b subjected to sublimation in vacuo at 100° C.		
a. Sublimate (crystalline)	0.50	0.036
b. Residue	0.22	>2

This was recrystallized three times from methanol and proved to be succinic acid:

	M.p.	Equivalent wt.	%C[2]	%H
Found	184.5°C.	60	40.8	5.40
Expected for succinic acid	185.0	59	40.7	5.08

Fraction 5b was next distilled at atmospheric pressure and yielded a series of fractions boiling between 60° and 150° C. Of these only the two highest boiling

fractions possessed any marked toxic activity. The residue from the distillation was then taken up in water and extracted with successive portions of benzene. The benzene extract on evaporation yielded a crystalline material which proved to be trans cinnamic acid:

	M.p.	% C	% H
Found.....	131°–133° C.	73.7	5.50
Expected for cinnamic acid.....	133°	73.0	5.40

It is to be noted that the total amount of activity present in the original steepate was not recovered in the acid-ether extract, or in this extract plus the residue. It may be that some loss of total activity accompanies this step. No procedure has as yet been devised which avoids this initial loss of activity. The fact that both of the active compounds obtained may be readily sublimed *in vacuo* at room temperature may account for this and other losses of activity.

Two highly active toxic compounds have been isolated from guayule root steepate—cinnamic acid and an unknown compound (4b). Since it has not been possible to isolate these compounds quantitatively, it cannot be stated with assurance how great a proportion of the total toxic activity of the acid-ether extract of the steepate is due to either. It would seem, however, that half or more of the toxic activity of the acid ether may be attributable to the compound 4b. Succinic acid, although somewhat toxic to guayule seedlings, accounts for only a negligible portion of the total toxic activity. Cinnamic acid is known, both in the free form and as the ester, from a wide range of plants, principally those containing essential oils or resins, as *Liquidambar* sp., *Styrax*, and *Cinnamonum* sp. The presence of cinnamic acid in the free form in the guayule plant has been reported by WALTER (19).

ACTIVITIES OF PURE COMPOUNDS.—As a result of the identification of cinnamic and succinic acids in guayule-root steepate, these substances—and related ones—were tested for toxicity to guayule seedlings (table 12). Cinnamic acid would appear to be a potent inhibitor of guayule seedling growth, one-half inhibition being given by 30 mg. per liter of the pure material under the test conditions. Figure 1 shows the relation between concentration and inhibition by cinnamic acid in the standard test. Cis cinnamic acid appeared to be at least as active as the trans isomer. It might be recalled that although cis cinnamic acid possesses auxin-like growth activity, the trans isomer is without such activity (5). Dihydrocinnamic acid (phenylpropionic acid) was five to ten times less toxic than trans cinnamic acid at the 50% inhibition level. It has been shown by WALTER (19) that a portion of the cinnamic acid in the guayule plant is present in the form of an ester with the sesquiterpene alcohol, partheniol. Parthenylcinnamate, a major constituent of guayule resin, is present in leaves, stems, and

TABLE 12

ACTIVITY OF PURE COMPOUNDS ON GROWTH OF GUAYULE SEEDLINGS

Substance	Concentration (mg./l.) needed for 50% inhibition in height growth
1. Trans cinnamic acid	30
2. Cis cinnamic acid	30
3. Dihydrocinnamic acid	220
4. Phenylacetic acid	130
5. Parthenylcinnamate	Saturated solution inactive
6. Partheniol	Saturated solution inactive
7. Melilotic acid	45
8. O-coumaric acid	45
9. Coumarin	350
10. Succinic acid	120
11. Fumaric acid	200
12. Malic acid	120

roots and is exuded from the roots of guayule plants. Both partheniol and parthenylcinnamate were nontoxic toward guayule seedlings.

The hydroxy derivative of cinnamic acid, o-coumaric acid, is active in inhibiting guayule seedling growth, and the hydroxy derivative of dihydrocinnamic, melilotic acid, is equally so. The lactone, coumarin, of o-coumaric acid, however, is practically inactive. Fumaric and malic acids, both related to succinic, have—as the latter compound—only low inhibiting activity.

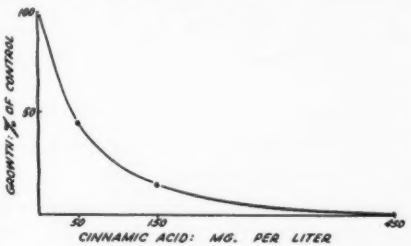

FIG. 1.—Influence of cinnamic acid on growth of guayule seedlings in unaerated solution culture. Growth given in percentage of that attained by control plants.

That cinnamic acid is inhibitory toward older guayule plants as well as toward seedlings is shown by the experiment of table 13. Nursery transplants, which had been topped nearly to the crown, were planted in 4-mesh gravel. The plants were supplied daily with nutrient solution, and in addition were watered once daily, either with pure water or with a solution of cinnamic acid. During the first 4 weeks as little as 1 mg. of cinnamic acid per liter significantly retarded growth, while at the expiration of 8 weeks significant reductions in dry-weight accumulation resulted from application of cinnamic acid.

Discussion

It has been shown that certain organic compounds which arise from guayule roots and accumulate in the solution surrounding such roots are definitely toxic to guayule plants. These toxic agents, although they were not isolated in pure form, were characterized chemically as being ether-soluble acidic compounds. The toxic agents which were isolated in pure form from water, in which guayule roots had been briefly allowed to steep, fulfil these criteria. There can be, however, no certainty that the compounds isolated are in fact identical with those which arise from normally growing guayule plants. It should be borne in mind, however, that at least one of the toxic agents, cinnamic acid, is a normal constituent of the guayule plant.

The mode of liberation of the toxic substances which arise from guayule roots has purposely not been discussed in the present paper, since it is impossible at

TABLE 13

INFLUENCE OF CINNAMIC ACID ON GROWTH OF GUAYULE NURSERY TRANSPLANTS; EXPERIMENT G-137

Solution	Height (cm.) after			Final dry weight (gm./plant)	
	4 weeks	6 weeks	8 weeks	Stems	Leaves
Control (no cinnamic)..	20.7±0.36	27.0±0.46	28.3±0.47	7.73	5.42
Cinnamic (1 mg./l.)....	18.2±0.42	25.8±0.47	27.0±0.41	6.86	4.40
Cinnamic (10 mg./l.)...	17.6±0.52	25.3±0.45	26.4±0.40	6.30	3.97
Cinnamic (100 mg./l.)..	15.8±0.57	23.4±0.59	24.8±0.56	4.93	3.82

the present time to state whether they are liberated from dead or injured cells, excreted by living cells, or arise in some other way. That the intervention of bacteria is essential is a thesis made unlikely in view of the fact that cinnamic acid is a normal constituent of guayule.

The toxic compounds studied have been shown to inhibit growth of guayule under certain limited environmental conditions. Whether or not the same compounds are active in the soil under field conditions in inhibiting and limiting growth of guayule is a separate question which remains to be investigated.

Summary

1. The experiments indicate that substances unfavorable to the growth of guayule plants emanate from the roots of actively growing plants of this species. These substances are organic in nature and can be extracted from nutrient solution which has previously been flushed through gravel cultures of guayule plants.

2. The growth-inhibitory extracts were assayed for their toxic activity by application to guayule seedlings grown in solution cultures under standard conditions.

3. The growth-inhibitory substances contained in nutrient solution which has been recirculated through guayule cultures are ether-soluble acidic compounds. They were not, however, isolated in pure form.

4. A potent source of growth-inhibitory substance was found in water in which guayule roots were allowed to soak briefly. From this water two compounds inhibitory to the growth of guayule plants were isolated in crystalline form: cinnamic acid, and a second unidentified organic acid.

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EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE RIPENING OF DETACHED FRUIT

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Introduction

Late summer applications of aqueous sprays containing 2,4-dichlorophenoxyacetic acid to woody plants such as sumac, sassafrass, and poison ivy resulted in marked changes in the color of the foliage of these plants. Sprayed leaves turned from green to red and yellow following treatment, in a manner similar to the coloration that occurs during the late fall. CHRISTOPHER (1) noted that attached fruit on apple trees which received preharvest "hormone" sprays developed a more intense color, and GERHARDT (2) observed that pears treated in a like manner ripened somewhat earlier than did unsprayed fruit. Furthermore, naphthaleneacetic acid and other growth-regulating substances have brought about an acceleration in the rate of starch hydrolysis when applied to the leaves of bean plants, a process which is associated with the ripening of certain kinds of fruit (8).

Experiments were therefore undertaken to determine the effects of growth-regulating substances on the rate of ripening of various kinds of fruit after removal from the plant. The effects of 2,4-dichlorophenoxyacetic acid on the ripening of apples, pears, bananas, tomatoes, peppers, and persimmons are reported here.

Methods

Fruits that differ widely in morphological and chemical constitution were used, namely: Grimes Golden, Yellow Newtown, and Rome Beauty apples; Keiffer and Winter Bartlett pears; bananas designated in the trade by the varietal names of Fortuna and Guatemala; Marglobe tomatoes; World Beater and Paprika peppers; and Miller persimmons. These fruits all exhibit physical changes during ripening which are readily measured—such as changes in color, solidity, starch content, or taste.

The bananas were obtained from a local wholesale market soon after arrival. All the other fruits were selected for uniformity of size and color and collected from the growing plants immediately prior to treatment. After treatment, the various lots of fruits were kept in well-ventilated rooms at 65°–70° F. At the time of treatment and at following intervals, the color of individual fruits as it changed was measured by means of a Department of Agriculture Standard Ground Color Chart for Western Apples and Pears. The red color of the individual fruits was estimated as a percentage of their total surface area.

The firmness of the flesh was determined on samples selected at random periodically during the ripening period, as measured by means of a U.S. Department of Agriculture fruit pressure tester. This instrument measured in pounds the force required to insert a blunt plunger, $\frac{3}{8}$ inch in diameter, into the flesh of the fruit of both apples and pears or through the peel and flesh of bananas.

Methods employed in treating fruits with 2,4-dichlorophenoxyacetic acid consisted of dips and sprays of aqueous solutions and also the liquefied-gas aerosol method. Aqueous solutions were pre-

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pared by first dissolving the required amount of compound in Carbowax 400, which served as solvent, carrier, and spreader. The required amount of this wax-hormone mixture was then added to a measured amount of hot tap water. Carbowax 400 was used in the aqueous solutions in concentrations of 0.1%, 0.5%, 1.0%, and 2.0% (10). The following concentrations of 2,4-dichlorophenoxyacetic acid were applied in aqueous solutions with Carbowax: 100, 250, 500, 800, 1000, and 1600 p.p.m. Dip treatments were made by completely immersing the fruit for approximately 1 second. Finely dispersed aqueous sprays were applied by means of compressed-air sprayers of 1-quart capacity that operated under 60–90 pounds of air pressure. In some instances localized applications were made with a pipette or small brush. Ten fruits each were treated in the following ways: (*a*) 1 ml. of solution was placed in the stem cavity of each fruit, (*b*) 1 ml. was placed in the blossom end (basin) of each fruit, (*c*) a narrow band $\frac{1}{4}$ inch in width was "painted" around each fruit at its greatest circumference, and (*d*) the entire surface was treated by immersion in the solution.

The method used in preparing liquefied-gas aerosols of organic compounds has been described by McGovran *et al.* (5). In treating by this method, an aerosol-forming mixture composed of 94% dimethyl ether, 5% cyclohexanone, and 1% 2,4-dichlorophenoxyacetic acid was made by weight. These mixtures were stored in cylinders of 1-pound capacity and were later sprayed on the fruits in the open air. Control lots were treated with a similar gas aerosol not containing the acid.

Statistical analysis of the data, both of the color readings and of the pressure tests on individual fruits, was by analysis of variance. Differences between treatments that exceed the 5% level are referred to as significant.

Results

Bananas

Stems of bananas were obtained on September 18 and on October 16 from storage rooms of wholesale dealers in Washington, D.C. The different lots of fruit had apparently received varied treatment with respect to conditions under which they had been grown and harvested, as well as under which they had been stored following harvest. Although the fruit on all stems was classified as "$\frac{3}{4}$ filled," they differed greatly in moisture content. The fruit obtained on September 18 was relatively fresh, as indicated by the fact that juice exuded readily from cuts made in the stem or fruit, while fruit obtained a month later was desiccated to the extent that juice did not flow from the cut surface when the stem was severed or the fruit freshly cut.

Fruit of the initial shipment, which was hard and deep green in color, was treated on September 18 as previously described. During the 24-hour interval immediately following treatment, those of the Fortuna lot to which 2,4-dichlorophenoxyacetic acid had been applied in concentrations of 200, 800, or 1600 p.p.m. changed from a deep green to a yellowish green color. Seventy-two hours after treatment, all the Fortuna fruits to which the acid solutions had been applied were completely yellow in color but firm to the touch, while the untreated fruits were hard and still deep green in color. Five days after treatment, fruits that received solutions containing 200, 800, or 1600 p.p.m. of the acid were ripe and of excellent flavor, while untreated fruits were still light green in color, bitter in flavor, and hard to the touch (table 1).

Iodine tests made on this date indicated that untreated fruits contained a much greater percentage of starch than did those to which solutions of the acid had been applied.

Ripening of the Guatemala variety was also hastened by application of 2,4-dichlorophenoxyacetic acid, but the time required for sprayed fruit to become entirely ripened was 1–2 days longer than for Fortuna. After all fruit had ripened, tests indicated that the quality of acid-treated fruit equaled, and in many cases exceeded, that of untreated fruit.

fruits were still hard and light green in color 9 days after treatment, while those to which the acid had been applied were completely yellow, or yellow with brown flecks, and fully ripe at the end of 5 days.

Relatively low concentrations (100 p.p.m.) were more effective in hastening ripening than were higher concentrations (1000 p.p.m.). Application of the acid by the aerosol method was slightly more effective than was the spray treatment.

Aqueous spray solutions that con-

TABLE 1

RELATIVE SOFTNESS OF FORTUNA BANANAS FOLLOWING SPRAY TREATMENT WITH 2,4-DICHLOROPHENOXYACETIC ACID. TREATED SEPTEMBER 18; PRESSURE READINGS 5 DAYS LATER

Concentration of acid (p.p.m.)	Averages of three readings per fruit (in pounds) Eight fruits per treatment								General average
Control	30*	30	30	30	30	30	30	30	30.00
200	13.3	12.0	13.6	14.0	11.0	14.0	16.6	12.3	13.37
800	11.3	12.6	12.0	14.6	12.3	13.0	16.0	12.3	13.33
1600	15.3	12.0	13.3	16.3	14.0	13.6	12.6	13.3	13.81

* Or over 30, the maximum for the tester used.

Fortuna bananas obtained on October 16 exhibited marked response to treatment with the acid, but ripening extended over a relatively long period of time, possibly owing to the fact that the fruit had been longer in transit than those of the previous experiment, or because they had been grown and harvested under a different environment. However, applications of solutions containing concentrations of 100 or 1000 p.p.m. greatly accelerated ripening (table 2). As in the earlier experiments, color change from green to yellow was associated with a decrease in hardness of the fruit and an improvement in its flavor, as indicated by taste tests made as the fruit ripened. Under the conditions of this experiment, the untreated

tained Carbowax 400 alone in concentrations of from 0.5 to 2.0% did not hasten the ripening of bananas. Solutions that contained 2,4-dichlorophenoxyacetic acid and 2% Carbowax were no more effective in hastening ripening than were others that contained only 0.5% of Carbowax and an equal amount of the acid. A weak alcoholic solution containing the acid was less effective in hastening ripening than was an aqueous solution of Carbowax that contained an equal amount of the acid (table 2).

APPLES

GRIMES GOLDEN.—Fruits that were still green in color were collected on September 12 and dipped in aqueous solutions containing 500 or 1000 p.p.m.

of the acid. They were almost fully yellow 6 days later. At the end of 11 days those treated with a lower concentration (250 p.p.m.) likewise had turned yellow (table 3). Comparable untreated fruit failed to color during this 11-day period, as indicated by color readings. Seventeen days after treatment, fruits in all treated lots were completely yellow in color, but fleshy parts of treated fruits were significantly softer in texture than those of untreated, as indicated by pressure tests.

In another experiment, freshly picked

TABLE 2

RELATIVE RATE OF COLORATION OF GREEN FORTUNA BANANAS FOLLOWING TREATMENT WITH 2,4-DICHLOROPHENOXYACETIC ACID. TREATMENTS APPLIED AS AQUEOUS SPRAYS AND BY AEROSOL METHOD, ON OCTOBER 16

TREATMENT	NO. OF FRUITS	AVERAGE COLOR READING				
		Oct. 19	Oct. 21	Oct. 23	Oct. 24	Oct. 25
Untreated control	41	1.00	1.80	2.10	2.50	2.75
2.0% Carbowax sprayed control	21	1.00	1.10	1.66	2.66	2.80
2.0% Carbowax; 100 p.p.m. of acid; sprayed	21	2.50	3.40	3.75	4.00	4.00
0.0%* Carbowax; 100 p.p.m. of acid; sprayed	21	1.00	1.75	2.22	2.80	3.00
2.0% Carbowax; 1000 p.p.m. of acid; sprayed	20	1.00	2.80	3.50	3.90	4.00
0.5% Carbowax; 1000 p.p.m. of acid; sprayed	23	1.00	2.66	3.40	3.90	4.00
2, 4-dichlorophenoxyacetic acid; aerosol	20	3.00	4.00	4.00	4.00	4.00

* Acid first dissolved in 0.5 ml. of 95% ethyl alcohol.

TABLE 3

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED IN AQUEOUS SOLUTION AS DIPS AND BY LIQUEFIED-GAS AEROSOL METHOD ON RELATIVE RATE OF CHANGE FROM GREEN TO YELLOW OF GRIMES GOLDEN APPLES. TREATED SEPTEMBER 12

TREATMENT	AVERAGE COLOR RATING OF TEN FRUITS				PRESSURE TEST IN POUNDS AV. OF TEN FRUITS AND SUM OF THREE READINGS PER FRUIT
	Sept. 18	Sept. 21	Sept. 25	Sept. 29	
Untreated control	1.5	2.3	2.2	2.6	42.0
Dipped control*	1.8	2.2	2.2	2.9	38.0
Dipped 250 p.p.m.	2.4	3.5	3.8	4.0	31.0†
Dipped 500 p.p.m.	3.1	3.5	3.8	4.0	29.5†
Dipped 1000 p.p.m.	3.6	3.6	3.9	4.0	30.6†
Aerosol without acid	1.8	2.1	1.9	2.8	41.0
Aerosol with 1% acid	2.1	2.7	3.4	4.0	32.3†

* All aqueous dip treatments contained 0.5% of Carbowax 400.

† Statistically significant difference from untreated controls at 5% level (difference of 8.64 required).

Grimes Golden apples were dipped in solutions containing 100 and 1000 p.p.m. of the acid on September 19. The fruits treated with 1000 p.p.m. were fully yellow 6 days after treatment, and were

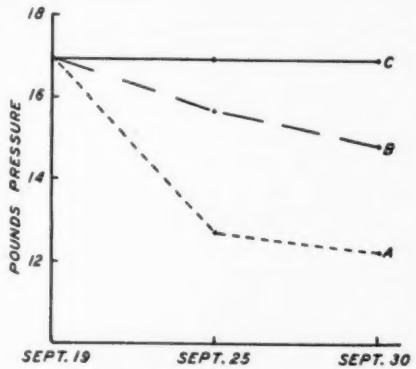

FIG. 1.—Rate of softening of Grimes Golden apples dipped: *A*, in solution containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid; *B*, in solution containing 100 p.p.m.; *C*, untreated fruits. Average pressure-test readings of ten fruits from respective treatments at each sampling date.

sweet in flavor and mellow (low pressure-test reading). Application of 100 p.p.m. of the acid likewise resulted in a statistically significant reduction in pressure-test readings as compared with those of the untreated fruits, at the end of both 6 days and 11 days following treatment (fig. 1). Final pressure readings obtained 17 days after treatment showed no significant difference between any of the treated or control lots. On this date, however, the controls were still relatively green in color (table 4) but had softened to about the same degree as the fruits in both treated lots.

It has been demonstrated repeatedly that the effects of application of growth-regulating substances to plants are often manifest at some distance from the point of application. An experiment was made to determine the effects of localized application of 2,4-dichlorophenoxyacetic acid on the rate of ripening of Grimes Golden apples. At the end of 11 days following treatment it was apparent from the standpoint of color that the ripening effect of treatment with the acid was localized. Treatment of the entire fruit, either by the dipping or the aerosol method, resulted in complete yellowing within 6–8 days following treatment. The fruits treated in localized areas were still green in color (rating 2 on color chart), except in those areas to which the acid solution was applied. These treated areas became yellow (rating 4 on color chart) and clearly defined within a period of 6–8 days following treatment. There was a suggestion, however, that treatments applied on the stem cavity had brought about a more general color change than had applications made on the blossom end (calyx) or around the greatest perimeter of the fruit.

TABLE 4

RELATIVE RATE OF COLOR CHANGE OF GRIMES GOLDEN APPLES FOLLOWING DIP TREATMENT IN AQUEOUS SOLUTIONS OF 2,4-DICHLOROPHENOXYACETIC ACID. TREATED SEPTEMBER 19

TREATMENT	AVERAGE COLOR READING PER TREATMENT				
	128 fruits	32 fruits	24 fruits	16 fruits	8 fruits
	Sept. 19	Sept. 22	Sept. 25	Sept. 30	Oct. 4
Untreated.....	1.7	1.9	1.6	2.3	2.9
Dipped control*...	.....	1.7	1.8	2.2	2.6
Dipped 100 p.p.m..	.....	1.8	2.0	3.0	3.1
Dipped 1000 p.p.m.	.....	1.7	2.2	3.6	4.0

* All dip solutions contained 0.5% Carbowax 400.

YELLOW NEWTOWN.—The effect of 2, 4-dichlorophenoxyacetic acid on the ripening of this variety of apple was de-

termined in order to compare the response of a late-fall variety with that of an early-ripening variety, such as Grimes Golden. Dip treatments at both 100 and 1000 p.p.m. concentrations applied on October 5 had no significant effect on pressure-test readings of the fruit at the end of an 18-day period following treatment. During the first 11 days following treatment, however, many of those treated with 1000 p.p.m. had developed a full yellow color, and at the end of 18 days practically all those treated with the 100 or the 1000 p.p.m. concentration were rated as full yellow in color (4 on color chart). In contrast, untreated fruits kept under the same conditions were still light green in color on the eighteenth day following treatment. Eighteen days following treatment the untreated fruits were sour and "starchy" in taste, while those treated with either 100 or 1000 p.p.m. were sweet and of good flavor.

ROME BEAUTY.—Fruits in the "mature-green" stage of maturity were carefully selected for uniformity of both red and green ground color. In general, the rate of softening and the changes in green ground color followed closely the results obtained with Grimes Golden. Twelve days following treatment, those fruits to which solutions containing 100 and 1000 p.p.m. had been applied were significantly softer than were untreated fruits. At the end of an 18-day period following treatment, the fruits in both treated and control lots were relatively soft (10–12 lb. pressure test). Color readings showed that the acid markedly reduced the intensity of the green ground color at both sampling days (11 and 18 days after treatment), and that the intensity of yellow color increased, thus greatly improving the appearance of the fruit. Eighteen days following treatment most of the untreated fruits were green and red, while the majority of treated fruits were yellow and red in color. Estimations showed that there was no appreciable difference in the percentage of red surface area on treated and untreated fruits.

PEARS

WINTER BARTLETT AND KIEFFER.—Although both of these are late-season varieties, they differ considerably in the manner in which the fruits ripen when harvested. Those of the Kieffer variety are usually harvested 7–10 days earlier, but the fruits generally remain firm for a relatively long period of time after being picked. Winter Bartlett pears usually soften very quickly under comparable handling conditions, even though the fruits may be much harder than Kieffer at the time of harvest.

Dip treatments, using aqueous solutions containing either 100 or 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid, hastened the rate of softening of fruits of both varieties, and treatment at 1000 p.p.m. on September 20 hastened the rate of softening of Kieffer pears so that there was no appreciable difference in the firmness of fruits of the two varieties when measured 2 weeks later (table 5). The rate of ripening of individual treated fruits varied less than did that of untreated fruits, as indicated by pressure and color tests made at the end of the experiment.

Winter Bartlett pears were so russeted that reliable color readings could not be obtained. Readings were obtained on Kieffer, however, at periodic intervals following treatment. Those treated with the 1000 p.p.m. concentration were almost fully yellow (3.3. on color chart) on the eighth day following treatment, while controls at this time were green in

TABLE 5

EFFECT OF AQUEOUS DIP TREATMENTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON RELATIVE RATE OF SOFTENING OF WINTER BARTLETT AND KIEFFER PEARS AS INDICATED BY PRESSURE TEST. TREATED SEPTEMBER 26

Concentration of acid (p.p.m.)	Pressure test in pounds, average of ten fruits					
	Oct. 3		Oct. 6		Oct. 10	
	Winter Bartlett	Kieffer	Winter Bartlett	Kieffer	Winter Bartlett	Kieffer
0 (Dry)	25.3	23.6	24.1	23.2	14.5	17.7
0 (Dipped)*	26.5	23.1	27.7	23.8	13.2	17.7
100 (Dipped)	26.5	21.0	15.4	18.6†	5.6†	9.6†
1000 (Dipped)	23.2	19.2	18.7	20.2	5.0†	5.1†

* Carbowax 400 used in all dipped lots at 0.5% concentration.

† Significant effect of treatment at 5% level (difference required: Winter Bartlett 7.18, Kieffer 4.66).

color (2.1 and 2.2 on color chart). At the end of 10 days following treatment, the treated fruits in both lots (100 p.p.m. and 1000 p.p.m.) were fully yellow (table 6). During the experiment, individual

TABLE 6

EFFECT OF AQUEOUS DIP TREATMENT OF 2,4-DICHLOROPHENOXYACETIC ACID ON RELATIVE RATE OF COLOR CHANGE BY KIEFFER PEARS. TREATED SEPTEMBER 26

Concentration of acid (p.p.m.)	Average color reading of fifty fruits			
	Oct. 3	Oct. 4	Oct. 6	Oct. 9
Untreated control	1.9	2.1	3.0	3.5
0*	2.2	2.2	3.0	3.8
100	2.6	2.6	4.0	4.0
1000	3.2	3.3	4.0	4.0

* Carbowax 400 used in all dipped lots at 0.5% concentration.

fruits of treated lots developed yellow color uniformly, while individual fruits of untreated lots varied widely in the rate at which they changed from green to yellow. This response was perhaps the most significant of any noted in connection with chemically controlled ripening of pears.

MARGLOBE TOMATO, WORLD BEATER PEPPER, PAPRIKA PEPPER, AND MILLER PERSIMMON.—Treatment with 2,4-dichlorophenoxyacetic acid, either as aqueous-solution dips or by the liquefied-gas aerosol method, failed to affect the rate of coloring or softening of any of these fruits. In all instances from ten to twenty mature green fruits were treated in the same manner and held under the same conditions that resulted in marked color changes and softening of such fruits as bananas, apples, and pears.

Discussion

The ripening of pears (2) treated with growth-regulating substances, such as naphthaleneacetic acid, to prevent preharvest drop is sometimes hastened; and apples, treated in a similar manner, may attain a more brilliant color than do other unsprayed ones on the same tree (1). Tomato fruits are known to respond in a similar way when treated with growth-regulators while yet attached to the vine (4). These effects may result from an increase in the rate at which materials are mobilized from other parts of the plant into the fruit (6), from the

activation or acceleration of chemical and physical processes associated with ripening (9), and possibly from an increase in the over-all vigor of the treated portion of the plant (7). In contrast, acceleration of the ripening processes of detached fruits must necessarily be associated with the activation or acceleration of chemical and physical changes which occur within the fruit. One such process, which is common to most starchy fruits during the ripening period, is the gradual conversion of starch to sugars. Based on results presented, diastatic activity in fruits such as apple, banana, and pear can be accelerated by the application of 2,4-dichlorophenoxyacetic acid. Fruits such as peppers, tomatoes, and persimmons, which are generally without an appreciable starchy reserve, failed to respond to treatment with this acid, as measured by their rate of color change during ripening. It remains to be learned whether the ripening processes of nonstarchy fruits, other than those concerned with external coloration, can be influenced by treatment with growth-regulating substance.

HALLER (3) has reported that the softening of apples during ripening is associated with the conversion of insoluble forms of pectin to soluble forms, thereby making the cell wall less resistant to pressure. Treatment with 2,4-dichlorophenoxyacetic acid may likewise have accelerated the rate at which this conversion process occurred in the pears and apples, thus accounting for the rapid decrease noted in the resistance of the tissues to pressure.

Through the use of growth-regulating substances such as this acid,[3] together with regulation of temperature, it may be possible to control to a greater extent the rate of ripening of fruits that have a starchy carbohydrate reserve by accelerating their rate of starch hydrolysis and color formation at the desired time. For instance, it is sometimes desirable to ripen fruits as soon as possible after harvesting, or after removal from storage. Based on responses observed in the present experiments, the use of 2,4-dichlorophenoxyacetic acid might also be of benefit in bringing about a more uniform rate of ripening among individual fruits, such as those of pears, which often ripen unevenly after harvest.

[3] 2,4-Dichlorophenoxyacetic acid is relatively nontoxic, since 200 mg. of the acid was fed daily to small experimental mammals with no apparent ill effects.

Summary

1. 2,4-Dichlorophenoxyacetic acid was applied to the detached fruits of bananas, apples, pears, tomatoes, peppers, and persimmons and its effect on the rate of ripening of these fruits observed. The fruits were collected while still unripe and sprayed with or dipped in aqueous solutions of the acid, which contained 0.5%–2.0% Carbowax. Other fruits comparable in stage of development were treated with the acid by the liquefied-gas aerosol method, and all were then stored at room temperature.

2. Based on color changes, the ripening period of bananas was shortened from 3 to 8 days following treatment. Treated fruits attained a full yellow color and excellent flavor, even though they were subjected to drier air and lower temperature conditions than is usually maintained in their commercial ripening. Yellow Newtown apples ripened during the 2 weeks' period immediately following treatment, whereas comparable untreated apples failed to ripen in this length of time. Grimes Golden apples treated with the acid approximately 1 week before the usual picking date, when

still green in color, ripened within a period of 6 days immediately following treatment. Untreated fruits picked at the same time required approximately 2 weeks at room temperature to reach the same stage of ripeness. Rome Beauty apples treated with the acid ripened 2–6 days earlier than the untreated ones, as indicated by both pressure tests and color readings.

3. The time required for Kieffer pears to change from green to yellow, when collected and treated approximately 10 days before the usual picking date, was 2–4 days less than required for comparable untreated fruits. Winter Bartlett pears ripened within a period of 8–10 days following treatment, while untreated fruits failed to ripen during this period. Treated fruits of both varieties of pears ripened more uniformly than the controls.

4. Based on color changes, the rate of ripening of fruits of tomato, pepper, and persimmon was not affected by treatment with the acid.

5. Starch hydrolysis proceeded at a more rapid rate during the ripening of treated bananas than it did during the ripening of untreated fruits, as indicated by both iodine and taste tests.

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SOME ANATOMICAL EFFECTS OF MOISTURE STRESS IN NURSERY SEEDLINGS OF GUAYULE

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Introduction

The effects of moisture on the development of guayule seedlings (*Parthenium argentatum* Gray) and their behavior subsequent to transplanting have been the subject of considerable study by the Soils Division of the Special Guayule Research Project. As part of this investigation, a limited study of the anatomical effects of moisture stress in nursery seedlings was undertaken.

MATERIAL AND METHODS.—Material was secured December 13, 1943, from experiments being conducted (5) at the Alisal Nursery, Salinas, California. A sample of ten plants was taken from each of three treatments: I, low moisture stress; III, intermediate moisture stress; V, high moisture stress.

The beds were seeded June 3, 1943, and all plants were given the same amounts of moisture until July 13, at which time the differential treatments were applied. Prior to seeding, the soil of all plots was wet to field capacity to a depth of at least 6 feet. In treatment I the moisture was kept between field capacity and a tension of 450 cm. of water at a depth of 6 inches until September 14. In treatment III it was attempted to keep the moisture between field capacity and a tension of 850 cm. at the 6-inch depth until August 30. Treatment V received no irrigation after July 13. The tension at lower depths in treatments I and III never exceeded that at the 6-inch depth. Rainfall between the end of moisture applications and the time of collection of samples was negligible.

Sections were made from (*a*) the root-crown, (*b*) a young stem, and (*c*) mature leaves of the plants in each sample. The root-crown was considered to be that portion of the axis derived from the hypocotyl; therefore this zone was the same age in all treatments. Sections from this region should be comparable, except for the pith, inner xylem, and primary phloem fibers; it was not possible to obtain sections showing similar degrees of transition in these tissues. Sections of young stem were taken from the internode showing the greatest elongation. It is uncertain whether these were comparable as to age, but since they had developed during the period in each treatment most favorable for growth in length, it was considered permissible to compare observations of the pith and other primary tissues which had matured immediately following elongation. Leaf sections were made from a band of tissue cut across the central portion of the leaf about 5 mm. wide. The leaves were those of recent development. They had all appeared after the differential moisture treatments had been terminated; hence it might not be anticipated that they would show many differences.

The material was fixed in formalin-propiono-ethanol and sectioned at 70 μ on a sliding microtome using a freezing attachment. The sections were stained with the oil blue-safranin-Congo red combination recently described (1). This staining showed the distribution of rubber and defined the principal tissues. Observations of stem, root-crown, and

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certain of those of leaves were made from sections stained with this combination. In addition, a portion of each leaf sample was imbedded in paraffin, sectioned, and stained with safranin-haematoxylin (4). Leaf measurements were made from these sections, as this stain permitted more detailed examination of leaf structure.

Measurements were made of four sections from each block of tissue, using an ocular micrometer. Calculations of areas were based on the following linear measurements:

Radius: from center of pith to outer edge of cork.
Pith: from center of pith to inner edge of xylem.
Xylem: from outer edge of pith to cambium.
Cortex-phloem: from cambium to phellogen. (For convenience and accuracy these two tissues were combined.)

In the root-crown, where the pith is in transition, the xylem measurements were taken from the center of the section to the cambium. The figures given for areas of xylem in the root-crown therefore include varying amounts of pith. The measurements were converted into percentage of area, and analyses of variance were made. Measurements of gross external characteristics—as well as the results of chemical analyses on the plants of these experiments—are being published by KELLEY *et al.*-(5).

Observations

The results of measurements are presented in tables 1–3. Table 1 summarizes the data from cross-sections of the root-crown. In general, the observations are as would be expected. The area of the sections increases with increasing availability of water. However, the development of each of the major tissues does not parallel the amount of water available to the plant. For example, the cork remains the same in each treatment with respect to both the total thickness of the layers and the width of the individual cells. The xylem and cortex-phloem show some interesting relationships. Whereas the thickness of the xylem increases directly with increasing availability of water, the development of the cortex-phloem tissues does not parallel that of the xylem. There is no significant difference between the cortex-phloem tissues in treatments III and V. Thus the increased amount of water available in treatment III over V is reflected in xylem development but not in cortex-phloem development. Under treatment I, which had the largest amount of available water, both xylem and cortex-phloem show significantly greater development than in the other two treatments. Apparently, under the conditions of treatment III, the development of xylem has priority over the development of cortex-phloem. ARTSCHWAGER (2) has made a similar observation relating to the tissues derived from the cambium as growth is resumed in the spring. During the first months of active growth he found that the development of xylem proceeds much more actively than the development of phloem.

For purposes of comparison, the proportionate areas of xylem, cortex-phloem, and cork were included in table 1. These figures, however, do not appear to be as illuminating as the absolute measurements.

At the level of the root-crown, the absolute width of the rays or the width in terms of number of cells is not affected by moisture stress.

The more narrow width of the cortical cells of plants grown under conditions of low moisture stress does not indicate that the cortex is small in this case. In view of the increased diameter of the

root-crown, the reduced width of the cortical cells appears to be caused by tangential stretching, and/or radial compression. Both of these were observed in the sections (figs. 1, 2). It may be pointed out in this connection that the cortex of guayule retains a low level of meristematic activity throughout the life of the plant. As the stem enlarges, radial divisions in the cortex maintain the integrity of the tissue. It is therefore possible to identify the cells derived from the primary cortex, even in the root-crown region of 13-year-old plants. This phenomenon is also known in other woody plants (3).

In considering the data of table 2, it should be recalled that the material from which these sections were obtained was the internode of the plant which showed the maximum elongation. They are therefore not comparable in all respects. These internodes developed during the time the treatments were being applied, however, and should be comparable with respect to their primary tissues. Measurements of total area, xylem, cortex-phloem, and cork, each of which may consist entirely or largely of secondary tissue, probably are of little value in this instance. They are included to indicate the general state of development of the material studied.

Among all treatments there was no significant difference in length of the cortical or epidermal cells. While the diameter of the pith in treatment I is considerably larger than in the other treatments, the number of cells along this diameter is not significantly greater.

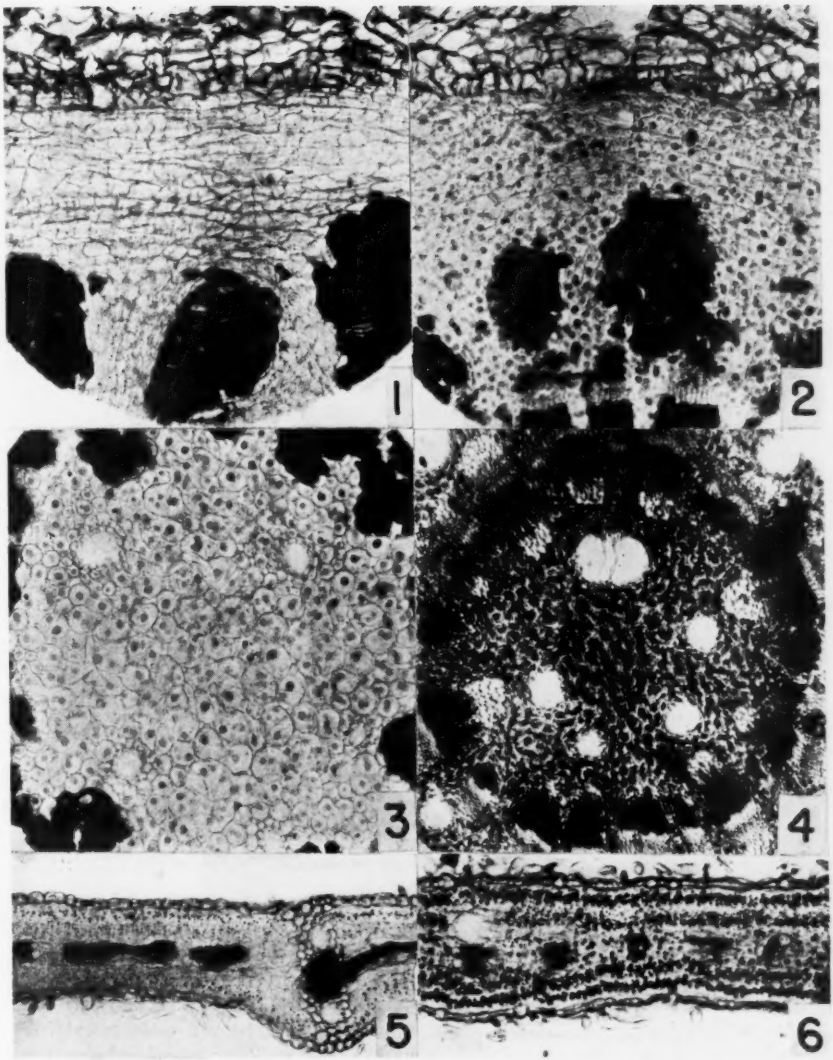
TABLE 1

MEASUREMENTS FROM CROSS-SECTIONS OF ROOT-CROWN OF PLANTS GROWN UNDER THREE DEGREES OF MOISTURE STRESS

	Moisture stress			Difference required for significance*	
	I Low	III Intermediate	V High	0.05	0.01
Total area of section	40.0 sq. mm.	21.1 sq. mm.	18.4 sq. mm.	5.2 sq. mm.	6.7 sq. mm.
Total radius of section	3.59 mm.	2.77 mm.	2.42 mm.	0.30 mm.	0.41 mm.
Width of xylem (radius)†	2.19	1.67	1.22	0.14	0.18
Width of cortex-phloem†	1.10	0.78	0.87	0.14	0.18
Width of cork†	0.30	0.33	0.33	Not sig.	Not sig.
Percentage of area:					
Xylem	37.0%	36.0%	25.5%	3.9%	5.5%
Cortex-phloem	47.0	41.5	49.0	3.0	4.2
Cork	16.0	22.5	25.5	4.3	5.8
Total	100.0	100.0	100.0		
Width of cork cells	0.077 mm.	0.071 mm.	0.073 mm.	Not sig.	Not sig.
Width of cortical cells	0.032 mm.	0.032 mm.	0.038 mm.	0.002 mm.	0.003 mm.
Width of vascular rays	0.049 mm.	0.048 mm.	0.048 mm.	Not sig.	Not sig.
No. of cells in ray	2.97	2.9	2.8	Not sig.	Not sig.

* If treatments differ more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the difference was not due to chance.

† An analysis of variance showed a highly significant interaction between tissues and treatments with a difference between differences of 0.19 required to give odds greater than 19:1 and 0.26 required to give odds greater than 99:1 against such differences being due to chance.



FIGS. 1–6.— Sections stained for rubber content, which appears as dark globules within parenchymatous cells. Figs. 1, 3, 5, from treatment I (low moisture stress). Figs. 2, 4, 6, from treatment V (high moisture stress). Figs. 1, 2, root-crown showing cork, cortex, and phloem. Figs. 3, 4, pith of young stem; note resin canals. Figs. 5, 6, leaves. Stem and root-crown sections are 70μ thick. $\times$80.

The increased diameter is therefore directly correlated with the larger diameter of the pith cells in treatment I.

The most striking effect of moisture stress in primary tissues of young stems is on the resin canals (figs. 3, 4). Treatment V, which received the least amount of moisture, had the largest resin canals. In this treatment the canals had more than four times the cross-sectional area of the canals in the plants that received the greatest amounts of moisture. This phenomenon appears to be related to the frequent observation that low amounts of available moisture lead to greater amounts of rubber and resins per unit of weight in guayule (7).

Several other effects were noted in the sections of the root-crown and of the young stem which could not be readily measured by the means at our disposal; nevertheless, they appeared consistently. As the available moisture was reduced, the amounts of rubber and lignin deposited increased. This effect was striking in the case of rubber, many of the sections from treatment V showing conspicuous amounts of stained rubber—even to the unaided eye. In the case of lignin deposition, the effects were less obvious but detectable in the more nearly complete lignification of the fibers in treatment V. This was less often the case as the available water was increased. As

TABLE 2

MEASUREMENTS FROM CROSS-SECTIONS OF YOUNG STEM OF PLANTS GROWN UNDER THREE DEGREES OF MOISTURE STRESS

	Moisture stress			Difference required for significance*	
	I Low	III Intermediate	V High	0.05	0.01
Percentage of area:					
Xylem	24.8%	23.6%	16.1%	4.9%	7.2%
Cortex-phloem	48.4	54.4	52.5	Not sig.	Not sig.
Cork	14.0	9.6	15.6	3.8%	5.6%
Pith	12.8	12.4	15.6	Not sig.	Not sig.
Total	100.0	100.0	100.0		
Total area of section	5.7 sq. mm.	3.1 sq. mm.	2.2 sq. mm.	0.8 sq. mm.	1.1 sq. mm.
Total radius of section	1.337 mm.	0.994 mm.	0.836 mm.	0.103 mm.	0.138 mm.
Width of xylem (radius)	0.347	0.248	0.145	0.051	0.067
Width of cortex-phloem	0.411	0.343	0.299	0.035	0.047
Width of cork	0.096	0.048	0.069	0.010	0.013
Length of cortical cells	0.043 mm.	0.042 mm.	0.045 mm.	Not sig.	Not sig.
Length of epidermal cells	0.073 mm.	0.063 mm.	0.068 mm.	Not sig.	Not sig.
Diameter of pith	0.937 mm.	0.690 mm.	0.660 mm.	0.092 mm.	0.123 mm.
No. of cells in diameter of pith	19.8	18.5	19.0	Not sig.	Not sig.
Av. diameter of cells in pith	0.047 mm.	0.037 mm.	0.035 mm.	0.005 mm.	0.006 mm.
Av. area of resin canals in pith	0.006 sq. mm.	0.012 sq. mm.	0.026 sq. mm.	0.012 sq. mm.	0.016 sq. mm.

* If treatments differ more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the difference was not due to chance.

would be expected, the increased deposition of rubber and lignin is closely correlated with the accumulation of carbohydrates within the plants (**8**).

Table 3 summarizes the measurements and counts from sections of the fully expanded mature leaves from the three treatments. In none of these did the leaves differ significantly. This is probably due to the fact that all treatments had gone without irrigation for 3 months prior to the time of sampling.

TABLE 3

MEASUREMENTS FROM CROSS-SECTIONS OF LEAVES GROWN UNDER THREE DEGREES OF MOISTURE STRESS

	Moisture stress			Difference required for significance	
	I Low	III Intermediate	V High	0.05	0.01
Leaf thickness	0.270 mm.	0.256 mm.	0.269 mm.	Not sig.	Not sig.
Length of parenchyma cells in midrib	0.170 mm.	0.181 mm.	0.172 mm.	Not sig.	Not sig.
Av. length of cells in mesophyll	0.045 mm.	0.042 mm.	0.042 mm.	Not sig.	Not sig.
No. of cells thick	8.0	8.1	8.4	Not sig.	Not sig.

Leaves which had developed under the differential moisture treatment had been lost, and the ones sampled had developed under closely similar moisture conditions in the soil. It is reasonable, therefore, to expect few differences in this material. It should be added, however, that the leaves from the three treatments showed great dissimilarity in the amount of rubber they contained. As in the stem sections, the lower the available moisture the more rubber was present (figs. 5, 6). It is possible that this was related to the amount of rubber in the plants of the different treatments. A relatively high concentration of rubber in the stem and root of a plant might lead to the accumulation of the precursors of rubber in the leaves and result in the increased deposition of rubber within them.

It is apparently difficult to modify the fundamental pattern of development in guayule. LLOYD (**6**) noted but little difference in the structure of leaves from plants grown under both extremes of moisture. ARTSCHWAGER (**2**) found that "plants of a given variety show little difference in sclerenchyma development under varying soil moisture stresses." This is an important observation, since the sclerenchyma fibers of the cortex-phloem region take up space which would otherwise be occupied by rubber-bearing parenchyma. The data presented here support and extend these findings. The thickness of the cork is not affected by moisture stress, nor is the width of the vascular rays. Neither is the length of cells in the cortex and epidermis affected, nor the width of cells in the cork and vascular rays. It may be, therefore, that these anatomical structures are controlled by genetic factors not readily influenced by the physiological condition of the plant.

Moisture stress did affect the size of the pith and the tissues laid down by the cambium. Low moisture stress leads to a larger pith and greater cambial activi-

ty. High moisture stress leads to greater deposition of rubber and lignin, as well as to marked enlargement of the resin canals. Most of these observations would be expected from a knowledge of the effects of moisture stress on the metabolism and growth of plants.

The effects of moisture stress on the rubber-bearing tissues of guayule are the aspects of this work which are perhaps of greatest interest. These tissues are the cortex, rays, pith, and certain regions of the secondary phloem; each is larger in treatment I (low moisture stress) than in treatment V (high moisture stress). It is interesting that in rubber-bearing tissues that are semimeristematic (as the cortex), and those derived from the cambium (the rays), cell size is not increased by low moisture stress but the number of cells is. In the pith, which is not meristematic, the effect of low moisture stress is to enlarge the tissue by enlarging the individual cells. The considerably larger size of plants grown under low moisture stress (5) indicates a much greater volume of each of these tissues than a comparison of cross-sectional areas would suggest. These observations on nursery seedlings corroborate and extend the findings of LLOYD (6) and ARTSCHWAGER (2) on field plants.

Summary

1. Sections of root-crown, young stem, and leaf of nursery plants of guayule that had been subjected to three levels of moisture stress were examined anatomically.

2. Under the conditions of the experiment, moisture stress had no effect on the thickness of the cork, the width of the vascular rays, the length of cells in the cortex or epidermis, the width of cells in the cork or rays, or on the number of cells along a diameter of the pith.

3. Low moisture stress leads to increased cambial activity, resulting in greater xylem and phloem areas with correspondingly longer vascular rays. The diameter of the pith and the size of its cells are also greater under low moisture stress.

4. At the intermediate moisture stress, the development of xylem appears to have priority over the development of phloem.

5. High moisture stress leads to increased deposition of rubber and lignin and to considerable enlargement of the resin canals.

6. In general, low moisture stress leads to enlargement of the tissues of the plant with corresponding increases in rubber-bearing capacity. High moisture stress leads to the accumulation of the products of photosynthesis.

Dr. H. M. BENEDICT generously aided with the statistical analyses.

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EFFECT OF CHEMICAL TREATMENTS IN PROLONGING DORMANCY OF TUNG BUDS. II

HAROLD M. SELL, HUGH A. TAYLOR, AND GEORGE F. POTTER[1]

Introduction

Injury to the flowers by late spring frosts is one of the most serious hazards in commercial tung-oil production in the United States. Losses in small localized areas occur almost every season, and a partial to an almost total loss of crop occurred throughout the tung-growing belt in 2 of the 6 years 1938–1943, inclusive. Although it is known that the trees tend to compensate for such losses through the production of an extra heavy crop after a nonbearing year, average production over a period of time is probably reduced at least 25% by frost damage.

Numerous investigators (**6–14**) have reported that certain synthetic growth-regulating chemicals affect the rate of growth of the terminal and lateral buds of plants. WINKLEPLECK (**12, 14**) found that α-naphthaleneacetic acid in a water solution, when sprayed on floral buds of peach trees prior to blossoming, retarded full bloom for 11 days. Later, MITCHELL and CULLINAN (**8**) extended this study to include the effect of indole-3-acetic acid, indole-3-butyric acid, and α-naphthalene acetamide—in lanolin or oil emulsions—on the opening of vegetative and floral buds of the peach and pear. In their work the expansion of the floral buds was not retarded except as a result of injury, although the compounds were applied to attached and to excised branches under different environmental conditions and to plant material which varied with respect to stage of development and physiological condition. In fact, under certain environmental conditions in the field they found that application of these substances may hasten the opening of floral buds. On the other hand, the growth of vegetative buds was consistently retarded as a result of the application of α-naphthaleneacetic acid.

In the tung tree, SELL *et al.* (**10**) observed in 1941 that dormancy was prolonged with α-naphthalene acetamide or indole-3-acetic acid and that there was a period of about 1 week during which injury to the buds on branches treated with the former by a frost of 28° F. would, according to FERNHOLZ (**3**), have been 5–15%, whereas on untreated branches it would have been 70–80%. In 1942 the study was extended to determine the effect of other organic compounds and also to determine whether ether and alcoholic extracts of tung buds contained any substance which would prolong dormancy. In many instances responses to the growth-regulating substances have been obtained through the use of a lanolin carrier (**7–10**). Since lanolin contains compounds of the steroid group and other organic substances, an effort was made to determine whether lanolin emulsion or some of the sterols and their palmityl esters in a partially hydrogenated vegetable-fat carrier might contain any biologically active substances that would prolong dormancy.

Recently, CLARK and KERNS (**2**) have established that flowering in pineapple

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could be induced in advance of the normal period—or considerably delayed—by the use of appropriate concentrations of such synthetic substances. Since the effect of concentration is such an important factor in controlling bud growth, the project of prolonging dormancy in tung was also extended to determine the effect of concentration of α-naphthalene acetamide and the effect of the date of its application.

In view of the injury resulting from application of growth-regulating substances reported by MITCHELL and CULLINAN (8) to buds of peaches and pears, a careful investigation was made to determine the extent of injury to the terminal tung buds.

Experimentation

EFFECT OF ORGANIC COMPOUNDS WITH VARIOUS FUNCTIONAL GROUPS

The term "tung buds" as used in this paper pertains only to flower buds, although leaf buds are also present in the flower cluster.

Thirty-one treatments were used, including the following as controls: untreated buds, Crisco (trade name for a partially hydrogenated vegetable fat) emulsion, and lanolin emulsion. In the twenty-eight other treatments, an emulsion—either of Crisco or of lanolin—was used as a carrier for the various substances to be tested. To compensate for the error due to the variation in data of bloom on individual seedling trees, the symmetrical incomplete block design as described by GOULDEN (5) was used. Each plot consisted of the buds (20–133) on a single branch. Six such branches were utilized on each of thirty-one trees 16 years old, making in all about 186 plots and providing six replications of each of the thirty-one treatments.

The six branches on a single tree constitute a "block" (235–387 buds); and in the symmetrical incomplete block design, adjustment for variations between blocks is accomplished by an arrangement of the treatments assigned to each block (in this case to each tree). For example, the six replications of treatment A were located on one branch on each of six trees, and since there were six experimental branches on every tree, the six trees on which treatment A was located had in the aggregate thirty-six experimental branches: six had been used for treatment A and the remaining thirty were available for other treatments; hence it was possible to apply the remaining thirty treatments (B, C, D, etc.), one on each of the remaining thirty branches. Thus it was possible to calculate from the data for these six trees (*a*) the average response to treatment A, and (*b*) the average response to all treatments. The blocks—that is, the groups of treatments assigned to each tree—are so arranged that what has just been described for treatment A holds true for every other treatment, and it is possible to compare the response to each of the thirty-one treatments in turn with the average response to all treatments on the same trees. Thus a common denominator that is independent of individual tree variations is provided for adjustment of the actual responses.

The various substances were mixed in a lanolin emulsion according to the method of WINKLEPLECK (13). The basic formula is lanolin 38.0 gm., stearic acid 7.5 gm., triethanolamine 2.7 gm., and water 100 gm. The substances to be tested were dissolved in the minimum quantity of dioxane and then stirred into the lanolin emulsion by means of a Hamilton-Beach dispersion apparatus. Concentrations of 0.50% in the emulsion

were prepared with the organic compounds and of 16% with the extracts of tung buds. For the preparation of a Crisco emulsion, Crisco was substituted for lanolin in the WINKLEPLECK formula.

Four applications of these various materials were made on February 12, 26, March 11, and 20, 1942, to the buds of individual branches on the various trees by means of a small varnish brush.

this by the total number of buds. For example, on the branch of tree 5 that had been treated with lanolin emulsion alone, there were on April 1 seven buds in stage 1, thirteen in stage 2, one in stage 3, and four in stage 4. Thus there were twenty-five buds, the sum of the products was fifty-two, and the average rating was 2.08. This figure (2.08) indicates that the average bud on the branch used as an

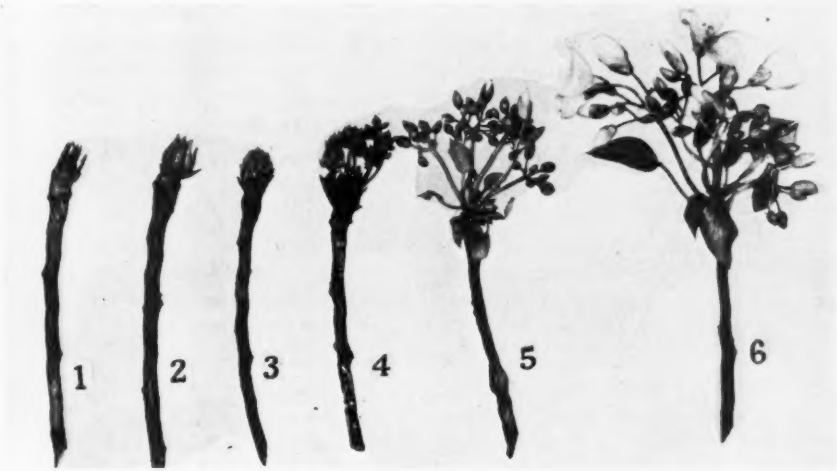

FIG. 1.—Tung buds at six stages of development

As in a previous study by SELL *et al.* (10), development of the buds from dormancy to post-bloom was divided into seven stages. The buds shown in figure 1 illustrate the midpoints of the first six stages. Stage 7 (not illustrated) comprises the terminal on which petals had begun to fall. When growth began, the stage of development of the living buds was assigned a numeral, rating from 1 (dormant) to 7 (post-bloom). The average rating of the stage of development of the buds on each branch was found by multiplying each numerical rating by the number of buds at that stage, adding the products, and dividing

example had developed slightly beyond stage 2 (fig. 1). Similar ratings were calculated for the five other control branches treated with lanolin emulsion, the average for all six branches was statistically adjusted as previously explained to compensate for the individual tree variation, and the adjustment rating 1.65 was entered in table 1 as the effect of lanolin-emulsion control on April 1.

As the season advanced, considerable injury to the buds was observed, and on May 30 the dead buds on each branch were counted. These data were expressed as percentages and have been analyzed

statistically after transformation to angles—as recommended by BLISS (1)—to obtain the F value (table 1). Since transformed data are of little interest, actual percentages of dead buds are shown in table 1. No standard error appropriate to the whole range of percentages shown can be calculated.

anhydride, 2, 4-dinitro-1-naphthol-7-sulfonic acid, para-aminobenzoic acid, 8-hydroxyquinoline, and ether and alcoholic extracts of tung buds. These substances neither caused greater injury to the buds nor retarded development more than did the lanolin-emulsion control Data for the average stage of development at weekly intervals and the percentage injury to the buds subjected to the remaining fifteen treatments are shown in table 1. Certain of these treatments were also without significant effect; but, owing to the chemical relationships of the compounds to either

TABLE 1

ADJUSTED RATINGS OF STAGE OF DEVELOPMENT (FIG. 1) AND PERCENTAGE INJURY FOR TUNG BUDS FOLLOWING FOUR APPLICATIONS OF 0.50% CONCENTRATIONS OF ORGANIC COMPOUNDS IN LANOLIN EMULSION (EXCEPT WHERE CRISCO IS INDICATED) MADE FEBRUARY 12, 26, MARCH 11, AND 20, 1942

TREATMENT	MARCH 19	MARCH 22	APRIL 1	APRIL 7	APRIL 13	APRIL 20	APRIL 27	DEAD BUDS AS OF MAY 30 (%)
	Adjusted ratings of stage of development							
Untreated control	1.42	3.17	3.98	5.66	6.77	7.00	7.00	0.00
Lanolin-emulsion control	1.02	1.16	1.65	2.99	4.21	5.75	6.82	7.49
Naphthalene	1.00	1.35	1.76	3.53	4.59	6.43	6.98	3.16
α-Naphthoic acid	1.00	1.00	1.06	2.71	4.02	5.84	7.00	12.52
α-Naphthaleneacetic acid	1.00	1.14	1.29	1.92	2.78	3.74	4.97	52.49
α-Naphthalene acetamide	1.00	1.00	1.00	1.61	2.90	3.74	5.22	38.52
α-Naphthalene thioacetamide	1.00	1.01	1.23	1.85	2.88	3.74	4.52	41.65
Indole-3-acetic acid	1.00	1.03	1.20	2.47	3.39	4.54	5.98	16.94
Phytosterol palmitate	1.03	1.09	1.29	2.85	4.37	5.74	6.91	12.91
Phytosterol	1.02	1.52	2.22	3.95	5.31	6.55	6.93	7.45
Cholesterol	1.08	1.32	1.60	3.29	4.36	6.30	7.00	7.33
Cholesterol palmitate	1.00	1.10	1.38	2.76	3.84	5.24	6.88	18.00
Crisco and phytosterol palmitate	1.03	1.50	1.99	3.70	5.12	6.83	7.00	1.33
Crisco-emulsion control	1.04	1.64	2.37	4.02	5.53	6.61	7.00	2.65
Crisco, cholesterol palmitate, and α-naphthalene acetamide	1.01	1.00	1.06	1.50	3.30	4.36	6.03	20.52
F for treatments*	4.15	8.72	9.90	9.36	9.78	7.64	3.94	5.70†
Standard error mean difference	0.054	0.20	0.26	0.37	0.38	0.48	0.49	‡
Least difference significant at 0.05	0.11	0.40	0.51	0.73	0.75	0.95	0.97	.....
Least difference significant at 0.01	0.14	0.52	0.68	0.97	1.00	1.26	1.28	.....
F required at 0.001 = 2.40								

* Value of F determined by analysis of data for thirty-one treatments, including seventeen not shown in table.

† Value of F obtained by analysis of transformed values.

‡ No standard error appropriate to whole range of percentages of dead buds can be calculated; for values approaching zero the error is smaller than for percentages approaching 50.

Data will not be presented for lanolin emulsions of the following compounds: 1,2-azonaphthalene, 2,4-dinitro-6-cyclohexylphenol, phenylthiohydantoic acid, sulfanilamide, ethyl carbamate, 3,5-dinitrosalicylic acid, sulfanilic acid, phenylisothiocyanate, pyridine, maleic

lanolin or naphthalene and its related compounds, the data are included in the table.

The lanolin-emulsion control retarded blossom development. By April 1, terminal buds on the untreated control branches had attained stage 4, in which they are rather susceptible to freezing injury, while those treated with the lanolin-emulsion control did not reach this stage until April 13—12 days later. According to FERNHOLZ (3), 70–80% of the pistillate flowers on terminals in stage 4 would be either injured or killed by a temperature of 28° F. However, the retardation appears to be the result of injury to the buds, of which 7.49% were killed.

In the series of naphthalene compounds (table 1) it was noticed that naphthalene produced no greater response than did the lanolin-emulsion control. A slight retarding effect was noticed with α-naphthoic acid on March 26, April 1 and 7, but it was only on April 1 that the response differed significantly from the lanolin-emulsion control. The α-naphthaleneacetic acid and its two derivatives gave the greatest response of any of the substances tested. On April 7, the control buds (untreated) and those treated with lanolin-emulsion control were nearly in stages 6 and 3, respectively, while those emulsions fortified with the α-naphthaleneacetic acid and its derivatives reached stage 3 on April 13, 6 days later. It was evident, however, that the increased retardation of development by α-naphthaleneacetic acid and its derivatives was associated with increased injury to the buds, since 38–52% of these were killed.

The indole-3-acetic acid was slightly less effective than the α-naphthaleneacetic acid derivatives. This may be due to the fact that the indole-3-acetic acid is a more unstable compound than the latter. Preparations of indole-3-acetic acid stored in amber bottles have been found to deteriorate on standing at room temperature in the laboratory for about 12 months. Purification of 3 gm. of this stored material yielded only 1 gm. having the correct melting point upon recrystallization from chloroform. Examination of the mother liquor showed that the remainder of the material had decomposed beyond recovery.

The lanolin emulsion—fortified, respectively, with cholesterol palmitate found in lanolin and phytosterol palmitate found in the wax of tung buds (table 1)—produced no response as compared with the lanolin emulsion without the growth-regulating substances. There is a slight indication that phytosterol palmitate in a Crisco emulsion retarded development early in the season more than the Crisco-emulsion control, but at no date does the difference attain statistical significance. However, phytosterol (Eastman grade) in lanolin emulsion produced significantly less effect in retarding bud expansion on April 1, 7, and 13 than did lanolin-emulsion control, but such differences were not significant on the other four dates. The Crisco-emulsion control prolonged dormancy about 1 week. Crisco emulsion fortified with cholesterol palmitate plus α-naphthalene acetamide yielded a significant response in prolonging dormancy but killed 20.52% of the buds. On March 26, the buds to which the Crisco-emulsion control had been applied were almost midway between stages 1 and 2, and this same stage was reached on April 7 by the buds treated with the Crisco emulsion fortified with cholesterol palmitate plus α-naphthalene acetamide. Since it has been shown previously that fortifying Crisco emulsion with choles-

terol palmitate did not increase its effect on dormancy, the response just noted can be attributed to the α-naphthalene acetamide.

The data in table 1 show that in all instances retardation of development of buds was associated with injury. The substances that prolonged dormancy for the longest time also killed the most buds. The palmityl esters of cholesterol and phytosterol produced a more moderate effect on growth and injured fewer buds. It is concluded that a 0.50% emulsion of the growth-regulating substance used in a lanolin emulsion is not a practical means of prolonging dormancy in terminal buds of the tung tree.

Effect of concentration and date of application of α-naphthalene acetamide

Two experiments were set up, one to determine the effect of different concentrations of α-naphthalene acetamide in single applications and the other to determine the effect of the same concentration of this substance used in schedules of repeated applications.

In the first experiment, lanolin emulsion made according to the formula of Winklepleck (13) was used in preparing three concentrations of α-naphthalene acetamide—0.50, 0.25, and 0.01%. On each of four dates (February 12, 26, March 11, and 20) these dilutions were applied by means of a small varnish brush to the buds of previously untreated branches of twenty-four seedling trees. Each treatment was replicated on six branches. Other branches on the same tree were left untreated as controls.

In the second experiment, the same concentrations of α-naphthalene acetamide were used in four schedules as follows: (*a*) four applications each on February 12, 26, March 11, and 20; (*b*) three applications on February 26, March 11, and 20; (*c*) two applications on March 11 and 20; (*d*) one application on March 20.

Under the same environmental conditions, different seedling trees vary widely in date of bloom. In order to correct for this source of error, the two experiments were arranged on a factorial basis, as described by Fisher (4). Each concentration was used on every date or schedule, with a control. These separate controls (untreated buds), although identical, are listed as separate treatments, thus giving sixteen treatments in each experiment. This permits the use of a simple yet adequate experimental design.

There were six replications of the sixteen treatments in each experiment. The degrees of freedom representing the interaction between concentration and date or schedule of application were partially confounded, thus permitting the sixteen treatments of each replication to be broken down into four blocks of four treatments each. The four branches of each of the twenty-four trees constituted a block. Thus, the variation in date of bloom of the individual tree had no effect upon the over-all responses (*a*) to date or schedule of application or (*b*) to concentration. This design also reduced to a minimum the effect of tree variation on the data for each specific combination of date with concentration.

At weekly intervals, beginning March 19, the stage of development of each bud on every branch was recorded as previously described. The average rating of the stage of development for each treatment on each date was calculated. The data show that the buds on control branches were about to blossom on April 8, therefore a statistical analysis has been made of the data for all treatments as of this date. The data on stage

of development and percentage of dead buds as affected by concentration at a certain date, and those for schedules of concentrations were applied on February 12 (table 2) had developed into stage 3 (fig. 1) by April 8, whereas—by repeated

TABLE 2

AVERAGE RATINGS OF STAGE OF DEVELOPMENT (FIG. 1) OF LIVING TUNG BUDS ON APRIL 8 AND PERCENTAGE OF DEAD BUDS AS OF MAY 30 IN RELATION TO CONCENTRATION, TIME, AND FREQUENCY OF APPLICATION OF α-NAPHTHALENE ACETAMIDE IN LANOLIN EMULSION

Dates of application	Percentage concentration of α-naphthalene acetamide								
	0.50		0.25		0.01		Control		Average (r)
	Dead buds (%)	Stage of development (r)*	Dead buds (%)	Stage of development (r)	Dead buds (%)	Stage of development (r)	Dead buds (%)	Stage of development (r)	
	Experiment 1, single applications								
2/12	.....	3.28	19.6	3.26	4.0	4.24	0.0	5.48	4.07
2/26	35.7	3.38	16.2	3.60	0.3	5.07	0.0	5.41	4.37
3/11	19.7	3.96	17.4	4.53	0.8	5.67	0.4	5.44	4.90
3/20	7.8	5.50	0.0	5.74	1.3	5.23	.....	5.52	5.50
Average	.....	4.03	.....	4.28	.....	5.05	.....	5.46	4.71
	Experiment 2, repeated applications								
2/12, 2/26, 3/11, 3/20 (schedule 1)	67.5	1.52	59.4	1.60	19.0	3.10	.....	5.42	2.91
2/26, 3/11, 3/20 (schedule 2)	63.4	2.07	39.4	2.05	11.9	4.46	0.0	5.17	3.44
3/11, 3/20 (schedule 3)	17.6	5.14	29.7	4.87	6.7	4.40	0.8	5.45	4.96
3/20 (schedule 4)	10.9	5.34	1.6	5.48	1.6	5.33	0.3	5.60	5.44
Average	.....	3.52	.....	3.50	.....	4.32	.....	5.41	4.19

	Treatments		Means	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Standard error of difference between ratings	0.34	0.32	0.17	0.16
Least difference significant at 0.05	0.68	0.64	0.34	0.32
Least difference significant at 0.01	0.90	0.85	0.45	0.43
Least difference significant at 0.001	1.18	1.11	0.59	0.55

No error applicable to whole range of percentage dead buds can be given.

* r = rating.

repeated applications, are summarized in table 2.

It is evident that the effects of repeated applications are cumulative. Buds to which the 0.50 or the 0.25% applications of the same concentrations (schedule 1, table 2)—development had been more effectively retarded and the buds were midway between stages 1 and 2. In general, however, repeated appli-

cations on all dates killed a much higher percentage of the buds than did single applications. These data add to the previous evidence that the prolonging of dormancy obtained in this way is associated with injury to the buds.

It is also evident that, to be effective, applications must be made early. Schedule 1, consisting of four applications (table 2), was the most effective in prolonging dormancy. No outstanding effect on date of bloom was produced by the two single-application tests made on March 20. The injury to the buds from applications made on this date was less than that produced by earlier applications. Although the two highest concentrations of the single applications of α-naphthalene acetamide applied on March 11 produced a statistically significant delay in date of bloom, this difference is not of practical importance since the buds required only about 1 day of growth to pass from stage 4 to stage 5.

For all dates and schedules of applications, without exception, there were no statistically significant differences in effectiveness in prolonging dormancy between 0.50 and 0.25% concentration of the α-naphthalene acetamide in lanolin emulsion. In the case of the single applications of February 26 and of March 11, the 0.50% concentration appears to have been slightly more effective than the 0.25%, but the difference is within the range of experimental error. The number of dead buds is somewhat greater with 0.50 than with 0.25%.

The 0.01% α-naphthalene acetamide when applied on February 12 was approximately one-half as effective as either the 0.50 or the 0.25% but produced no effect when applied on February 26. In schedule 1, consisting of four applications beginning February 12, the 0.01% concentration again proved to be approximately one-half as effective as either the 0.50 or 0.25% concentration. In schedules 2 and 3 of the repeated applications, this 0.01% concentration was less effective than in schedule 1. It produced very little injury to the buds, excepting in schedules 1 and 2.

In 1942, the period of dormancy[2] in tung buds terminated about mid-March, and the optimum time of application was about 1 month earlier. Applications had to be repeated to attain maximum effectiveness. Of the concentrations used in this experiment, 0.25% of α-naphthalene acetamide in lanolin emulsion proved the most effective. Injury to the buds occurred wherever blossoming was delayed, however, and no combination of concentration with date of application was found satisfactory or practicable for delaying blossoming.

Summary

1. Dormancy in tung buds was prolonged by treatment with lanolin emulsion alone, the buds remaining about 12 days longer in a stage not injured by a temperature of 28° F. This treatment killed 7.5% of the buds. Crisco emulsion was approximately one-half as effective as the lanolin emulsion and produced less injury.

2. Alpha-naphthaleneacetic acid, two of its derivatives, and indole-3-acetic acid were the only compounds used in lanolin emulsion that prolonged dormancy more than did the lanolin emulsion alone. The percentages of dead buds resulting from these more effective treatments ranged from 12.5 to 52.5, whereas the percentage for the lanolin emulsion was 7.5.

[2] The term "dormancy" as used in this paper does not refer to the rest period but rather to that period during which no development of the buds was observed in the field.

3. Repeated applications of 0.50 and 0.25% of α-naphthalene acetamide in lanolin emulsion were more effective than single applications in prolonging dormancy and also killed more buds. The injury was slightly greater with the 0.50% concentration. No significant differences in response were noticed between the 0.50 and 0.25% concentrations. The 0.01% concentration was approximately one-half as effective as the 0.50 or the 0.25% and produced considerably less injury. The indications are that two applications of 0.25% α-naphthalene acetamide in a lanolin emulsion made about 30 and 15 days prior to bud expansion would give best results in prolonging dormancy. Since considerable injury occurred whenever blossoming was materially delayed, no combination of concentration with date of application was found practical for orchard use.

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2,4-DICHLOROPHENOXYACETIC ACID AS A DIFFERENTIAL HERBICIDE

PAUL C. MARTH[1] AND JOHN W. MITCHELL[2]

Introduction

In studying plant responses to growth-regulating substances, it has since 1936 been observed repeatedly that some of these substances are toxic to plant tissues when applied in relatively large amounts. Thus, localized application of lanolin paste containing 2% or more of indoleacetic acid or of naphthoxyacetic acid often results in the death of tissues near the treated regions; and in connection with vegetative propagation of plants, overtreatment with these compounds has often resulted in greatly reduced growth or in death of the cuttings. For some years, responses to growth regulators, which were associated with decrease in plant growth, were considered disadvantageous in crop improvement, and they received relatively little attention. However, KRAUS (3) in 1941 proposed that the growth-inhibiting properties of these compounds be utilized in connection with weed control.

Recently, experimental evidence was obtained indicating that 2,4-dichlorophenoxyacetic acid, a potent substance whose activity was discovered by ZIMMERMAN (6), possesses herbicidal properties when applied in a solution of Carbowax (5) or when dispersed as a liquefied-gas aerosol (1, 2).

In April, 1944, experiments were undertaken at Beltsville, Maryland, to determine the sensitivity of various common weeds to treatment with 2,4-dichlorophenoxyacetic acid. These experiments have since been extended in order to determine the possibility of using the acid as a differential herbicide, and the results are reported here.

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Methods

Two methods of application were used, aqueous sprays and the liquefied-gas aerosol. The aqueous spray solutions were prepared by dissolving the required amount of the acid in Carbowax 1500 (polyethylene glycol), then adding this solution, with stirring, to the required amount of tap water. The Carbowax served as a carrier and spreading agent (5) and was added in amounts sufficient to give a 0.5% solution. Sprays were applied to plants or small plots with a precision sprayer of 1-quart capacity and carrying an air pressure of 60–100 pounds, or by means of a knapsack 3-gallon sprayer when somewhat larger plots were treated.

In dispersing the acid as an aerosol, a metal cylinder equipped with outlet and valve was evacuated. One hundred fifty grams of dimethyl ether and 25 gm. of engine oil (SAE 40) containing 3.15 gm. of 2,4-dichlorophenoxyacetic acid was drawn into the vessel. The mixture was then dispersed as an aerosol by releasing the valve, and treatments were applied by directing the mist toward the plants for several seconds. In some instances the plants were first covered with a cloth tent and the aerosol released within the enclosure, where it was allowed to remain for a period of several seconds.

PRELIMINARY EXPERIMENTS.—In preliminary experiments under field condi-

tions, weeds on plots of ground 2 × 2 feet were treated with the aerosol and the behavior of the plants later compared with that of others of like species on an adjacent untreated plot. In other exploratory experiments, weeds of uniform size and in the seedling stage were transplanted from the field to 4-inch pots and grown under greenhouse conditions until well established. The species used included *Plantago lanceolata*, *P. major*, *Datura stramonium*, and *Capsella bursa-pastoris*. Aerosol treatments were applied to these plants in varying amounts and their subsequent behavior noted.

EXPERIMENTS WITH DANDELION.—On September 2, an area of Kentucky bluegrass lawn 32.5 feet long and 13 feet wide was selected in which an even, dense stand of dandelion (*Taraxacum officinale*) was growing. Six adjacent plots 6.5 feet square were laid out, two replications for each of three treatments. The following treatments were applied: (*a*) untreated control, (*b*) 500 p.p.m. of 2,4-dichlorophenoxyacetic acid applied as a spray at the rate of 4 gallons per 1000 square feet, and (*c*) 1000 p.p.m. of the acid applied in the same manner and rate. The spray treatments were repeated 26 days after the initial treatment. On September 21, Milorganite fertilizer was applied at the rate of 20 pounds per 1000 square feet of lawn surface. During the experiment the lawn was cut at regular intervals and given usual lawn care.

EXPERIMENTS WITH PLANTAIN.—On August 28, an area of Kentucky bluegrass lawn heavily infested with narrowleaf plantain (*Plantago lanceolata*) was selected for uniformity of both grass and plantain. Three parallel tiers of plots 6 feet square were laid out in this area, with twenty plots in each tier—a total of sixty plots. A 1-foot border was left around each plot. The three tiers of plots were separated into four equal blocks or replicates, each being five plots long and three plots wide. Aqueous sprays containing 2,4-dichlorophenoxyacetic acid in concentrations of 0, 125, 250, 500, and 1000 p.p.m. were applied. Five plots in each block were selected at random, and on August 28 four of these received a single spray treatment at each respective concentration level. Five other plots in each block received two applications of the respective solutions, the first on August 28 and the second on September 7. The five remaining plots in each block were reserved for treatment during the spring season.

All spray applications were made by means of a 3-gallon knapsack sprayer at the rate of approximately 4 gallons per 1000 square feet. The grass was cut when needed during the experiments and the lawn given usual care.

Results

PRELIMINARY EXPERIMENTS.—Plants of *Datura*, *Plantago*, and *Capsella* transplanted from outdoors and grown under greenhouse conditions during April were found to be very sensitive to application of 2,4-dichlorophenoxyacetic acid. Seedlings of *Datura* were killed by aerosol treatments of 5 seconds' duration. Exposure for 1 second to the aerosol resulted in the death of plants of *Plantago* (fig. 1) and *Capsella*. These treatments were applied after the plants had developed visible flower buds but before any flowers had opened. Within 48 hours following treatment the plants showed severe leaf-curl, and—in the case of *Datura*—stem bending. There was no evidence of burning of the leaves, but growth of the plants was inhibited, the leaves became light green in color, and within 3 weeks the sprayed plants were dead. European bindweed (*Convolvulus*

arvensis) grown from seed during early spring under greenhouse conditions was also killed by a 5 seconds' exposure to the aerosol.

In preliminary field experiments, treatment applied on August 11 to mature dandelion plants resulted in complete killing by August 29. Likewise, applications of aqueous sprays containing 1000 p.p.m. of the acid resulted in almost complete eradication of several hundred dandelions from a lawn area 35 feet long and 10 feet wide. Two plots containing forty-five to fifty plants each of ragweed were treated on August 12 by means of the aerosol method, while two similar adjacent plots received a thorough coverage with an aqueous spray containing 800 p.p.m. concentration of the acid. Periodic examination of these plots indicated that application of 2,4-dichlorophenoxyacetic acid, both as an aerosol and in water, had resulted in complete inhibition of vegetative growth. Maturation of flowers was inhibited as the result of both methods of treatment, and the shedding of pollen was prevented. On August 29, all the plants in the aerosol plots were dead. Ninety per cent of the plants that received the aqueous-spray treatment were dead at this time, and the remainder were severely distorted. After death of the ragweed, volunteer crabgrass present at the time of treatment rapidly covered bare areas in all plots.

FIG. 1.—Eradication of narrow-leaf plantain (*Plantago anceolata*) with 2,4-dichlorophenoxyacetic acid applied by aerosol method: *1*, untreated; *2*, exposed to the aerosol for 1 second; *3*, for 5 seconds; and *4*, for 20 seconds. Photographed approximately 1 month after treatment.

EXPERIMENT WITH DANDELION.—A more detailed experiment was undertaken, as previously described, to determine the effectiveness of 2,4-dichlorophenoxyacetic acid when used to eradicate dandelion plants in a Kentucky bluegrass lawn. The spray treatments listed in table 1 were applied to the treated plots on September 2 and the original number of dandelions per plot determined 3 days later. On the third day following treatment, the plants sprayed with the acid were severely twisted and had turned from dark green to a pale yellowish green color.

TABLE 1

ERADICATION OF DANDELION IN KENTUCKY BLUEGRASS SOD BY AQUEOUS SOLUTIONS OF 2,4-DICHLOROPHENOXYACETIC ACID AT 500 AND 1000 P.P.M. CONCENTRATIONS. FIRST SPRAY APPLICATION ON SEPTEMBER 2; SECOND ON SEPTEMBER 28

SPRAY CONCENTRATION (P.P.M.)	NO. OF DANDELION PLANTS PER PLOT (AV. OF 3 PLOTS)			PERCENTAGE ORIGINAL PLANTS KILLED	
	Sept. 7	Sept. 26	Oct. 19	Sept. 26	Oct. 19
Unsprayed control	118.0	213.5	215.0	0	0
500	94.6	22.0	3.0	75.6	95.8
1000	117.0	4.6	1.5	96.0	98.3

During an interval of 24 days following treatment, the 500 p.p.m. spray reduced the original number of dandelion plants by 75.6%, while 1000 p.p.m. reduced the dandelion population by 96.0% (table 1; fig. 2). The number of

dandelion plants in control plots more than doubled during the same interval, owing to unrestricted germination of the seeds. Later examination showed that many of the dandelions killed by the spray treatments had completely disintegrated as the result of attacks by soil organisms. Apparently, application of killing as obtained previously by a single application of a spray containing 1000 p.p.m. of the acid (table 1). On October 19, the newly planted grass had germinated and was growing vigorously. The bare areas had been almost completely covered with grass in the reseeded portions of the treated plots.

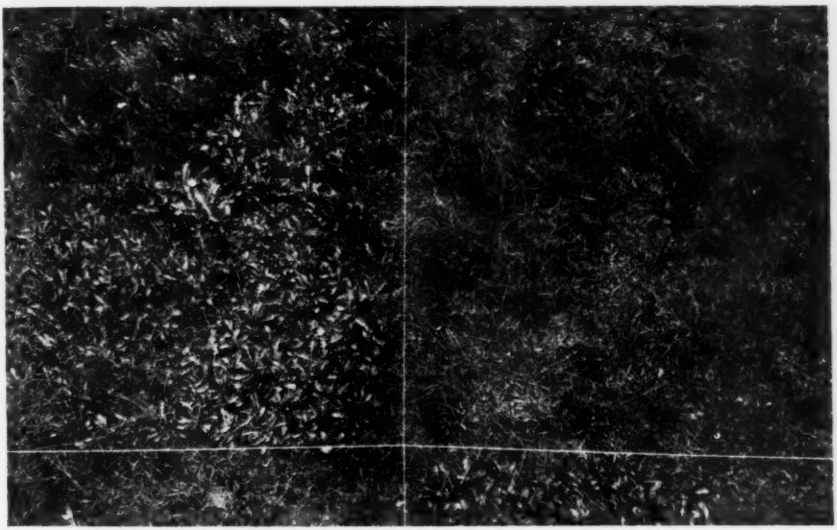

FIG. 2.—Eradication of dandelion from Kentucky bluegrass sod by spraying at rate of 4 gallons per 1000 square feet of sod with aqueous solutions of 1000 p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax. Sprayed plot at right; untreated control plot at left. Spray applied September 2 and plot photographed 24 days later.

the acid did not greatly inhibit the subsequent development of microörganisms in the tissues after the plants died.

Eradication of the dandelions left numerous bare areas, but the grass continued to grow following treatment, and regular weekly mowings were necessary to maintain it at a height of 1½ inches. The plots were resprayed and one-half of each plot was reseeded on September 28. Dandelion counts were again made on October 19. Retreatment with the 500 p.p.m. concentration resulted in approximately the same percentage of

EXPERIMENT WITH NARROW-LEAF PLANTAIN.—On August 28, experiments were undertaken, as previously described, to determine the effectiveness of 2,4-dichlorophenoxyacetic acid when used to eradicate narrow-leaf plantain growing in an area of well-established lawn. Heavily infested plots sprayed twice with a solution containing 500 p.p.m. of the acid, or that received either one or two applications of 1000 p.p.m., were practically free of plantain 3 weeks after treatment (94.6–99.0% eradicated). There was no appreciable change in the

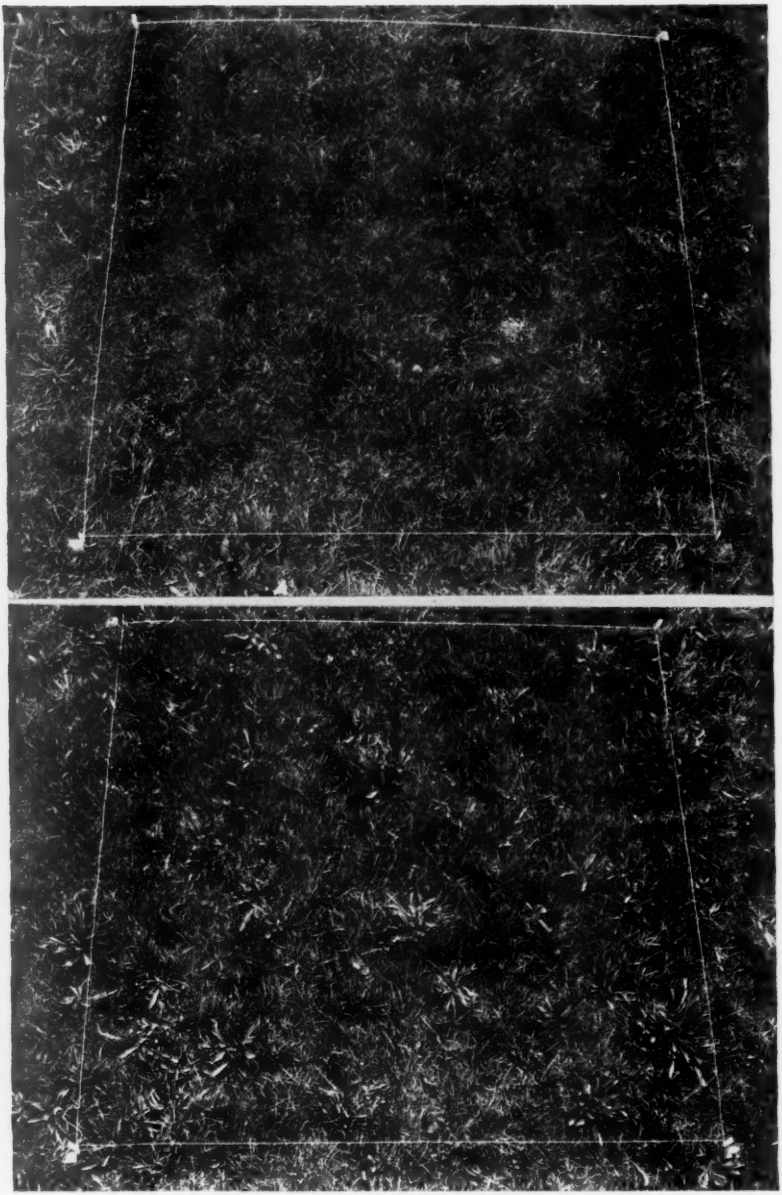

FIG. 3.—Eradication of narrow-leaf plantain from Kentucky bluegrass sod. Above: aqueous solution of 1000 p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax. Below: untreated control plot. Spray applied August 28 and plots photographed 23 days later.

population of plantain in untreated plots during the 52 days immediately following the first treatment, at which time final readings were made (table 2; figs. 3, 4). Lower concentrations (125 and 250 p.p.m.) were less effective, and some of the injured plants appeared to recover in these treatments. It is noteworthy that a single treatment at the 500 p.p.m.

TABLE 2

EFFECT OF ONE AND OF TWO AQUEOUS SPRAY APPLICATIONS OF VARIOUS CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID IN ERADICATING NARROW-LEAF PLANTAIN IN PLOTS 6×6 FEET OF KENTUCKY BLUEGRASS SOD

Spray concentration (p.p.m.)	No. of applications*	No. of plantain plants per plot (av. of 4 plots)			Percentage original plants killed	
		Original count on Aug. 28	Count on Sept. 29	Count on Oct. 19	Sept. 29	Oct. 19
Unsprayed control	0	62.4	66.1	69.7	0	0
125	1	68.2	69.0	64.0	0	0.6
	2	58.7	42.7	41.0	22.3	20.9
250	1	52.5	47.2	39.5	10.0	24.7
	2	54.2	18.5	26.5	65.9	51.1
500	1	53.7	14.5	17.2	73.0	67.6
	2	63.0	3.5	0.7	94.0	98.8
1000	1	45.5	2.0	3.0	95.6	93.4
	2	50.2	0.5	0.2	99.0	99.5

* Single spray treatment on August 28; duplicate sprayings on August 28 and September 7.

concentration killed only 67.6% of the plantain, while the double application at this concentration was as effective as the 1000 p.p.m. spray applied either once or twice. These data indicate that, although thorough coverage may be of considerable importance, it was also necessary to deposit the acid on the weed foliage in sufficient amounts per unit surface area to bring about adequate selective killing. Apparently, repeated sprayings with relatively low concentrations would give about the same effects as fewer applications of sprays containing relatively high concentrations, provided approximately equivalent amounts of the acid are deposited in each instance. Further experiments are necessary to determine which type of spray treatment is more desirable. At present, grass in all the plots is growing vigorously. Grass that received a spray containing 1000 p.p.m. is a somewhat darker, richer green than that in other plots which received the more dilute sprays. Intensification of green coloration has also been noted with other experimental plants, such as bean and tomato, when these were sprayed with solutions containing growth-regulating substances. Whether in this instance it may or may not be desirable has not yet been determined.

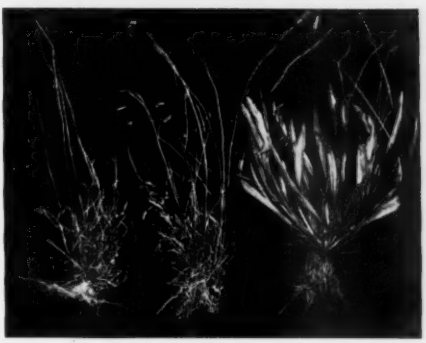

FIG. 4.—Effect of 2,4-dichlorophenoxyacetic acid (1000 p.p.m. spray) on mature field-grown plants of plantain. Left: two applications, August 29 and September 7. Center: single application, August 29. Right: untreated control.

A number of common weeds other than those already listed occurred in the treated plots in many of the experiments. The following were very sensitive and readily killed by aqueous sprays at 500–1000 p.p.m. concentrations: woodsorrel (*Oxalis* sp.), chickweed (*Stellaria media*), pigweed (*Amarantus retroflexus*), knotweed (*Polygonum aviculare*), and morning glory (*Convolvulus sepium*).

Other weeds—such as oxeye daisy (*Chrysanthemum leucanthemum* var. *pinnatifidum*), yarrow (*Achillea millefolium*), broad-leaf plantain (*Plantago major*), sheep sorrel (*Rumex acetosella*), dock (*Rumex obtusifolius*), as well as two species of *Rubus*—a blackberry and a trailing dewberry—appeared to be more resistant to the sprays and tended to recover from single applications at 1000 p.p.m. concentration. Preliminary results, however, indicate that these weeds may be killed by repeated sprays at this concentration.

A series of similar experiments reported elsewhere (4) has been in progress in co-operation with the Greens Section, United States Golf Association. These concern the eradication of Dutch white clover from bluegrass and bentgrass sods. In these experiments clover was found to be very sensitive to aqueous sprays containing 2,4-dichlorophenoxyacetic acid (fig. 5). In the same series of experiments, shiny pennywort (*Hydrocotyle rotundifolia*), which had been crowding out the bluegrass in a lawn for a period of several years, was found to be even more readily killed than clover. A single spray with the 500 p.p.m. concentration of the acid at 5 gallons per 1000 square feet resulted in its complete eradication from bluegrass sod, without apparent injury to the grass.

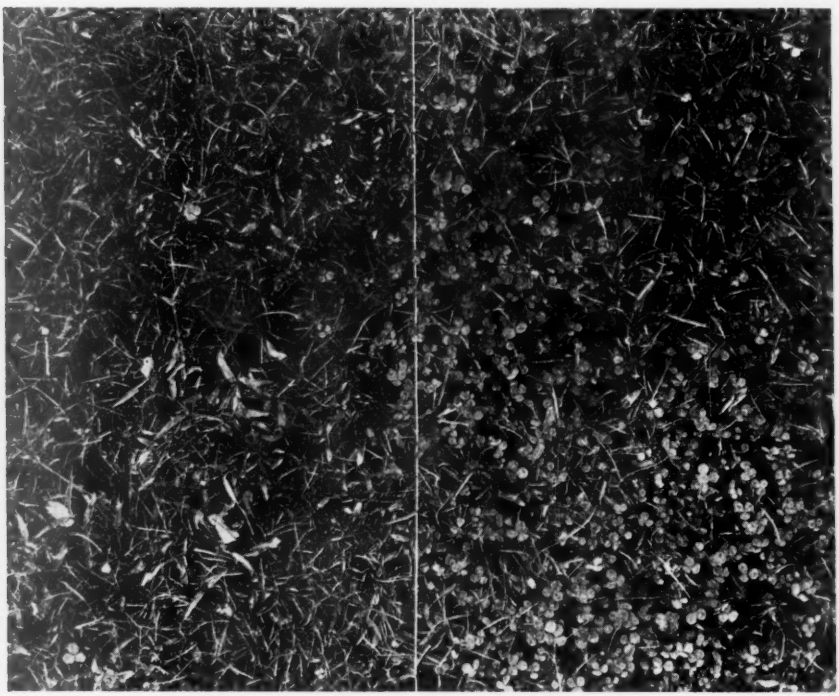

FIG. 5.—Eradication of Dutch white clover from Kentucky bluegrass sod. Left: 1000 p.p.m. concentration of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax applied as spray in water. Right: untreated control. Bluegrass and crabgrass in treated plot apparently uninjured.

Discussion

Chemicals such as sodium chlorate, sodium thiocyanate, and various arsenical preparations have been used extensively for weed eradication during the last decade. These compounds are extremely toxic to plants when applied in relatively large amounts (1–10% solutions), and their killing action is generally characterized by a localized burning effect which may later extend through the plant for some distance from the point of contact. In contrast, 2,4-dichlorophenoxyacetic acid is effective in concentrations of approximately 0.025–0.1%. It does not cause rapid superficial burns, but it brings about morphological and physical responses at the point of application or in parts of the plant at some distance from this region. These growth responses usually occur soon after treatment (within 24 hours), and apparently before the concentration of the acid or of its derivatives reaches a lethal level in the plant, and they are characterized by distorted stem and leaf growth, by inhibition of bud growth (particularly the terminal ones), and sometimes by the formation of galls or roots in the main stem. These responses are followed by death of the plant and disintegration of its tissues. Succulent plants that are susceptible to treatment with the acid often exhibit distorted growth for a period of 2–3 weeks following treatment, and they eventually die and are then readily digested by soil microörganisms.

There are marked differences in sensitivity between closely related plants when treated with 2,4-dichlorophenoxyacetic acid. For instance, *Plantago lanceolata* is extremely sensitive to treatment, while *P. major* is much less so. Bluegrass and crabgrass were relatively insensitive, while certain closely cut bentgrasses were relatively sensitive. Little is known concerning the biological effects of growth-regulating chemicals when applied to soil in varying amounts. Accordingly, caution should be exercised in treating lawn or pasture areas until further information has been obtained as to the relative sensitivity of various kinds of plants and the effects of the compounds when present in the soil.

Summary

1. 2,4-Dichlorophenoxyacetic acid was effective as a differential herbicide when applied as an aqueous spray in concentrations of from 250 to 1000 p.p.m. or more.

2. Plants killed by these sprays were dandelion, narrow-leaf plantain, Dutch white clover, chickweed, pigweed, wood-sorrel, knotweed, broad-leaf dock, bindweed, and shiny pennywort. Other plants, such as broad-leaf plantain, sheep sorrel, daisy, yarrow, and various species of *Rubus*, were relatively insensitive to the acid.

3. Two applications of either 500 or 1000 p.p.m. concentration of the acid in aqueous solution were made to well-established Kentucky bluegrass sod without apparent injury to the grass. In addition, Kentucky bluegrass seed planted under a light top dressing of soil, which was then sprayed with the acid concentrations, germinated and readily became established in these lawn areas.

4. It was possible to obtain 95% control of dandelion and narrow-leaf plantain by a single spray application of a solution containing 1000 p.p.m. of 2,4-dichlorophenoxyacetic acid or with two

applications at 500 p.p.m. concentration.

5. Caution should be exercised in the use of sprays containing the acid until more information is obtained concerning the effects of its presence in the soil.

Appreciation is expressed for assistance and helpful suggestions from L. W. KEPHART.

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SELECTIVE HERBICIDAL ACTION OF MIDSUMMER AND FALL APPLICATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID[1]

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Introduction

In a previous paper (7), attention was called to the herbicidal action of 2,4-dichlorophenoxyacetic acid on bindweed, following suggestions by KRAUS (9) and MITCHELL and HAMNER (10). Mention was made of its effect upon several other species of plants, and suggestions were made for its use as a herbicide. The present paper discusses additional results when this acid is applied as a water spray on bindweed in cultivated areas; on sow thistle in a garden plot; on mixtures of common weeds in the field and in a bluegrass lawn; and on several woody plants. It also presents results with the aerosol method of application (5) and with the residual effect in the soil as evidenced by seed germination on treated areas. Some of the results are fragmentary but seem worth presenting at this time.

MATERIAL AND METHODS.—All trials were conducted in the immediate vicinity of Geneva, New York, between July 14 and October 14, 1944. The soil of the area is characteristically heavy, has a pH of 6.5, and is provided with a fairly uniform moisture supply. The region is adapted to a wide range of horticultural and agronomic crops.

The season of 1944 was one of generally favorable growing conditions, with the possible exception of a period of drought and higher-than-normal temperatures during July. Rainfall and temperatures are given in table 1. Light frosts occurred on September 30 and October 16 and 17; the first killing frost was on October 20.

All trials were with 2,4-dichlorophenoxyacetic acid, secured from the

[1] Journal Paper no. 605 of the New York State Agricultural Experiment Station.

American Chemical Paint Company. Unless otherwise noted, it was applied in a water spray with a 5-gallon knapsack sprayer at a concentration of 1000 p.p.m., after first being dissolved in Carbowax 1500 at the rate of 1 part of the acid to 5 parts of Carbowax 1500, as described by MITCHELL and HAMNER (10).

TABLE 1

RAINFALL, MEAN MAXIMUM, MEAN MINIMUM, AND MEAN TEMPERATURES FROM JUNE TO NOVEMBER, 1944, AT GENEVA, NEW YORK

	June	July	Aug.	Sept.	Oct.
Rainfall (inches)	3.96	1.72	2.37	2.43	1.75
Temperature (°F.)					
Mean maximum	80.0	86.0	87.0	76.0	64.1
Mean minimum	57.2	59.8	60.9	52.5	39.6
Mean	69.0	73.2	73.9	64.2	51.9

I. Results with application as a water spray

BINDWEED

Bindweed (*Convolvulus arvensis* L.) is one of the most noxious weeds in the United States (11). It reproduces freely from seeds and also very rapidly from underground roots and rhizomes. Roots may penetrate to depths of 10 feet (1, 3, 8), although the main horizontal roots are usually just above the watertable (8), and rhizomes may arise 30–59 inches below the surface of the soil (1). To test the effect of 2,4-dichlorophenoxyacetic acid on this weed, the following experiments were carried out.

EXPERIMENT 1.—In the preliminary report on the action of 2,4-dichlorophenoxyacetic acid on bindweed (7), the chemical was applied on July 14 to two 100-foot rows of apple nursery stock in an area so infested with this weed that it was felt it might have to be abandoned. The temperatures for several days both preceding and following the application were approximately 80°–85° F. by day and 55°–60° by night. No rain fell for several days, either before or after application, and general field conditions were dry. Ten days after treatment, the above-ground parts of the plants were spongy, water-soaked, and enlarged to twice the diameter of similar parts from unsprayed plants. They soon decayed, at least to a distance of 14 inches below the surface.

Portions of roots and rhizomes dug from both treated and untreated plants were placed in a propagating frame to test their ability to regenerate. The portions from untreated plants made new growth (fig. 1), while those from treated plants failed to regenerate and died.

Following this response, nursery rows in an area of about 2½ acres were sprayed on July 31. One 600-foot row was left as a control. The area between the rows, which could be frequently cultivated, was not sprayed. The temperature was about 89° and the atmosphere dry. Within a few hours after the spray was applied, the field was disced and the soil thrown up to the rows of nursery stock so that it covered the bindweed with about 3 inches of moist soil. Ten days after treatment, the leafy parts of the bindweed were dead and the portions which are normally underground were decaying. The plants in the untreated areas between the rows showed many fresh shoots arising through the layer of soil which had covered them.

On November 9, when the soil was plowed away from the treated rows, no shoots or roots of bindweed were found to the depth of the plow; they had decayed and disintegrated. On the other hand, in the untreated row abundant live shoots and roots were uncovered in plowing, and vines were twined about the nursery stock.

EXPERIMENT 2.—On August 3, in order to test the method of entry of the chemical into the plant, the tips of individual plants of bindweed in the field were placed in vials containing 20 cc. of spray solution. Ten plants were used, three growing points or tips of each plant being placed in each of the ten vials. For comparison, about 3 inches of the tips of ten other plants were covered to protect them from spray and the uncovered portions of the plants then sprayed with the same material. The responses were similar from both methods of application. Ten days after application all above-ground parts were dead, and below-ground parts were proliferated and split as previously described.

EXPERIMENT 3.—On August 21, an area of about 2000 square feet containing a dense patch of bindweed was sprayed, but in this instance the solution was applied warm (110° F.). The area had been under frequent cultivation and the only weed growing was bindweed. It was in an active state of growth, and many young shoots had only recently emerged from the ground. The effect of treatment was pronounced. The tops were completely and uniformly killed, all at about the same time, whereas in other treatments not employing warm water some of the plants lingered a day or two after others were dead.

EXPERIMENT 4.—On September 9, on a relatively cool day (55° F.), about fifty bindweed plants were treated in a gar-

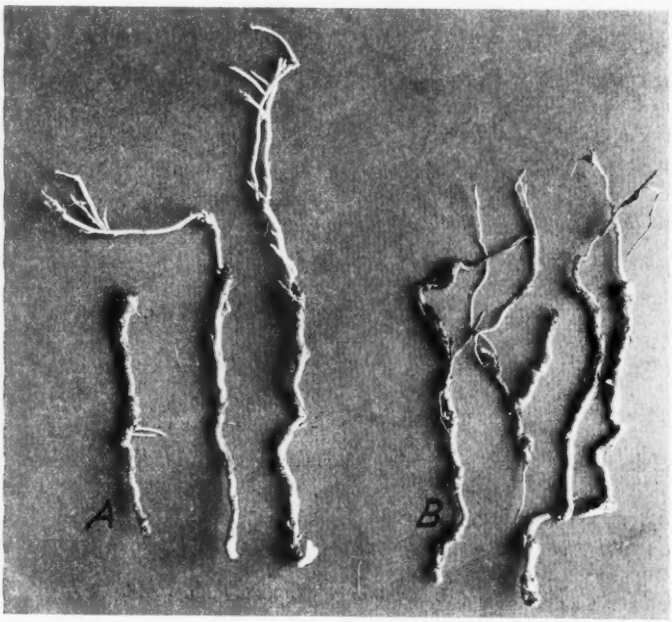

FIG. 1.—Effect of foliage spray on regeneration of roots and rhizomes of bindweed: *A*, untreated; new shoots developing. *B*, treated; proliferation, splitting, and decay.

den plot. The air temperatures following the treatment were lower than those following the preceding treatments, with several cool nights (45°–55° F.). The response was much slower, and complete killing of tops occurred only after 3 or 4 weeks.

Sow thistle

Sow thistle (*Sonchus arvensis* L.) is also a difficult weed (11) which regenerates rapidly from root pieces. To test the effect of 2,4-dichlorophenoxyacetic acid, a garden area was selected which was heavily infested. The garden had been hoed and cultivated during the early summer, so that the plants had been cut about 1 inch below the crown. Many root pieces had been severed and had apparently regenerated into a vigorous and persistent mass of plants. Individual plants were 12 inches in diameter, with leaves 10 inches in length.

The plot was divided into two sections, each consisting of about 200 square feet. On September 9, one section was given a very thorough foliage spray; the other left untreated. The weather was cool (about 55°–60° F.) and rain had fallen for 2 days. After treatment the temperatures were 55° F. at night and 70°–80° during the day.

Within 24 hours of treatment, the plants appeared lighter in color and lacked turgidity. Within 2 days the younger leaves showed severe twisting and curling. After 10 days the treated plants were still lighter green than the controls, and the outer row of leaves was flat on the ground, with a decidedly wilted appearance. The central group of leaves showed extreme curvature. The midribs near the base were enlarged and ribbon-like, and the bases were much enlarged and flattened, resembling celery stalks. The surface area occupied by individual treated plants was reduced by 60%.

The roots of the treated plants were greatly enlarged. An increase of at least 50% in diameter was common, and in some instances it was 300%. The roots were soft and spongy, showing much splitting and disintegration of the outer parts.

On September 25, 2 weeks after treatment, the untreated plants were completely dead, both tops and roots.

Mixture of common weeds in field

Applications were made variously to five areas on which were growing a range of weed plants common to the region, all of which are more or less of an economic problem. Most of them were at or near the flowering stage. Responses were much the same for all treatments, with a few exceptions that will be noted, so that only typically representative responses need be given:

1. Uncultivated orchard; mixture of twenty-two grasses and common weeds; 25 × 25 feet. Treated July 25; temperature 70° F., slightly cloudy, slight breeze blowing.

2. General farm land, cultivated until June 1; mixture of twenty-four grasses and common weeds, with germinating sweet clover; 24 × 36 feet. Treated August 21; temperature 75° F., sunny.

3. Headland of fertile truck farm; dense mat of purslane and pigweed, with scattering of seven other common weeds; 10 × 6 feet. Treated August 21; temperature 75° F., sunny.

4. Edging of stone driveway; mat of chickweed, dandelion, plantain, and grasses; 25 × 4 feet. Treated August 26; temperature 70° F., clear, sunny; light wind.

5. Rows of cultivated nursery trees; scattering of pigweed and purslane;

several hundred feet. Treated July 14, 80°–85° F., dry; and July 31, 89° F., dry.

In general, the greatest responses under these treatments were in those areas and under those conditions most favorable to plant growth. That is, the least effect was noted in the uncultivated orchard where plants were in a less active state of growth and the greatest in cultivated areas of good fertility. Further, responses were least during hot, dry conditions of July and with the more mature plants. At such times and under such conditions only the more active and less mature parts of the plants were affected—as tips, flowers, and below-ground meristematic parts. Later, when growing conditions were more favorable, as in late August, the responses were much more intense and affected a greater portion of the plant.

The responses of individual species, however, were characteristic:

Agropyron repens Beauv. (quack-grass); more than 100 plants.—No visible effect.

Amaranthus retroflexus L. (pigweed); fifty plants.—Within 3 hours main stem bent at 75–90 degree angle 6 inches from tip, becoming stiff; curvatures in petioles and leaves; at 1 day bending and stiffening of main stem progressing downward to within 2 inches of base on some plants, petioles stiff and recurved; at 4 days plants chlorotic, dry, woody, flower bracts brownish, flowers arrested, stems showing enlargement and splitting; at 29 days 50% of plants dead, remainder with a few tip leaves still functioning, rotting and blackening pronounced near split portions of stems (fig. 2).

Ambrosia artemisiifolia L. (ragweed); twenty plants.—Within 3 hours racemes showing severe curvature and becoming pendant (fig. 3); at 2 days all terminal growing points arrested; at 4 days plants becoming progressively dry and brownish; at 29 days all plants brown and dead.

Asclepias syriaca L. (milkweed); ten plants.—Within 1 day second and third pairs of large leaves near apex of plant folded upward against main stem; at 2 days third and fourth pairs of large leaves folded upward, main stem bent to horizontal, bending progressing downward; at 9 days splitting, bending, and breaking of stems of four of ten plants just above ground, latex no longer present in plants examined; at 29 days unbroken plants recovering (fig. 4).

Avena fatua L. (wild oats); ten plants.—No visible effect.

Chenopodium album L. (lambs-quarters); thirty plants.—Within 1 day plants wilted in appearance, stems recurved at 2 inches from tip; at 2 days flowers and terminal growing points arrested, minor curvatures of stems, plants becoming stiff and woody; at 29 days stems split in many places, plants very rigid and woody, all dead.

Digitaria ischaemum Schreb. (small crab-grass); more than thirty plants.—Suggestion of swelling at nodes but no striking response.

Digitaria sanguinalis Scop. (large crab-grass); more than thirty plants.—Suggestion of swelling at nodes but no striking response.

Echinochloa crus-gallia Beauv. (barnyard-grass); ten plants.—No visible effect.

Eleusine indica Gaertn. (goose-grass); ten plants.—No visible effect.

Lycopersicum esculentum Mill. (tomato); two plants.—Within 1 day leaves folding upward along midrib, leaf stems curving downward, base of petioles enlarged; at 7 days leaves chlorotic, much enlargement of nodes, stems, and bases of petioles, terminal growing points arrested; at 29 days nodes greatly en-

larged, typical formative effects, root primordia on stems, leaves becoming brown, leaf area reduced 90% (fig. 2).

Malva rotundifolia L. (round-leaved mallow); twenty-five plants.—Within 3 hours leaves curved downward; at 1 day stems recurved at 3 inches from tips; at 2 days leaves becoming chlorotic, land bare of the clover in contrast with adjacent field with abundant stand.

Plantago lanceolata L. (buckhorn plantain); three hundred plants.—Within 3 hours strong curvature of younger leaves; at 2 days leaves slightly chlorotic, roots with water-soaked appearance, discolored (fig. 5); at 10 days outer older

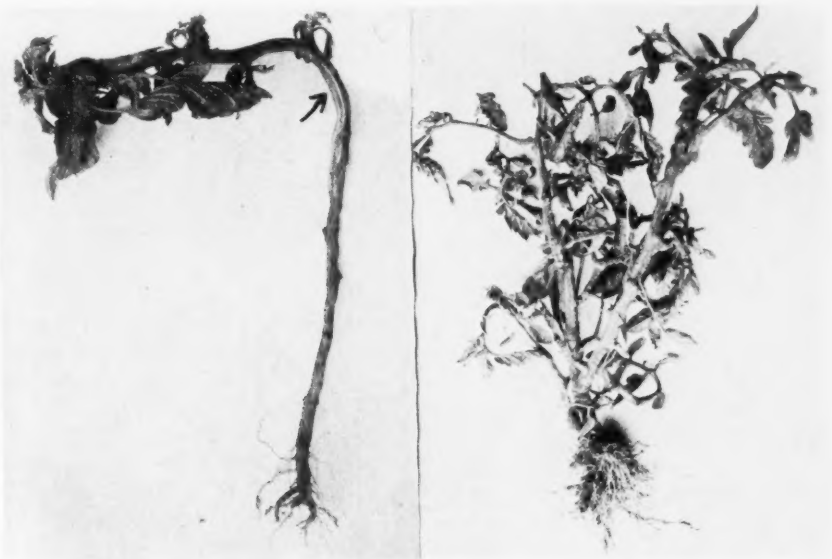

FIG. 2.—Effect of foliage spray on pigweed and tomato: Left, pigweed showing severe curvature and splitting of stem (arrow). Right, tomato showing enlarged stems, root primordia, swellings at nodes, curvatures, and arrested development.

flowers and terminal growing points arrested; at 13 days stems and parts of roots split, some callous formation in wounds, plants very stiff and woody, fifteen plants dead (fig. 3); at 29 days all plants dead and disintegrated in tops and roots.

Melilotus alba Desr. (white sweet clover); several thousand seedlings emerging 3–7 days after treatment to soil.—Severe spiral twisting of plants and downward folding of leaves as plants emerged, complete killing; at 29 days leaves flattened to ground, younger flower stalks white, elongate and curled, old flower stalks brown, central younger leaves curled, bases of leaves whitish, thickened, crinkled, ribbon-like, base of leaves and upper root sections rotting, complete absence of new flower stalks; at 30 days all plants dead, tops and roots disorganized and disintegrated.

Plantago major L. (broad-leaved plantain); fifty plants.—Within 1 day epinasty of leaves and petioles, young flower spikes recurved; at 2 days base of

leaves white, elongate, flat, ribbon-like; at 4 days mature flower spikes brown and drying out, young spikes etiolated; at 29 days 50% of plants dead, others

Polygonum pennsylvanicum L. (Pennsylvania smartweed); ten plants.—Within 1 day plants severely flattened to ground; at 2 days flowers checked in

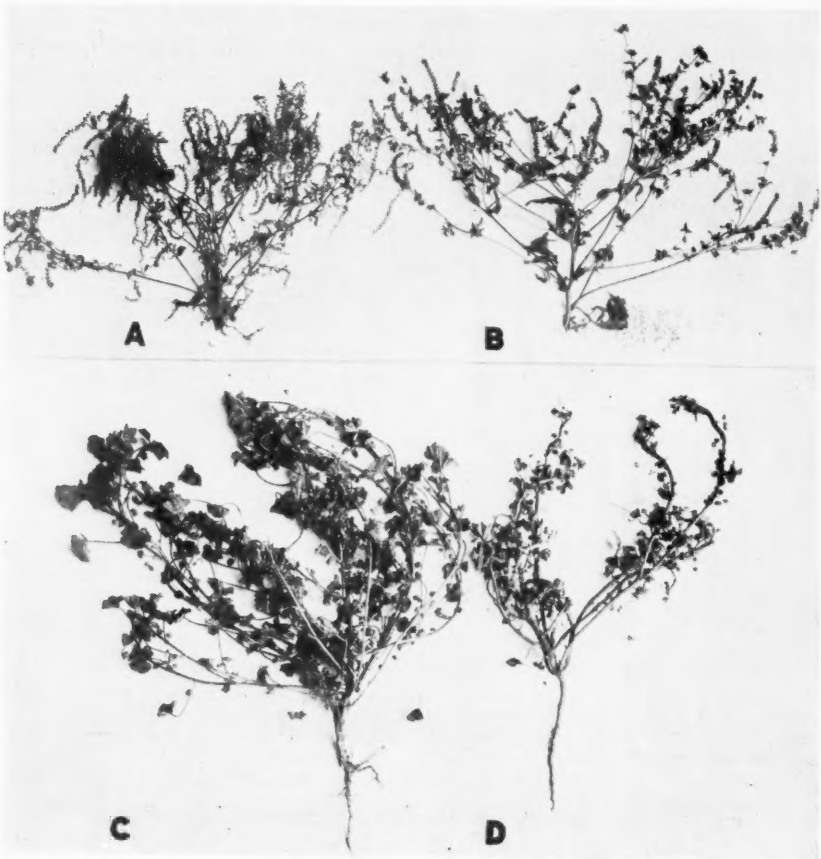

FIG. 3.—Effect of foliage spray on ragweed and round-leaved mallow: *A*, ragweed, sprayed, showing severe curvature of racemes. *B*, ragweed, untreated. *C*, round-leaved mallow, untreated. *D*, round-leaved mallow, sprayed, showing severe check in growth and flower development.

alive but with many torn leaves, dead flower stalks, and no new growth of either flower stalks or vegetative portions.

Poa pratensis L. (Kentucky bluegrass); more than 1000 plants.—Within 10 days darker green color, no visible injury.

development; at 29 days all plants dead.

Portulaca oleracea L. (purslane); two hundred plants.—Within 3 hours leaves becoming recurved; at 1 day tendency of plants to cling to ground, stems brittle; at 2 days foliage and stems chlorotic; at 7 days some leaves abscising, stems

very brittle; at 29 days most plants still alive, stems cracking and splitting, decay entering wounds, stems easily broken, almost all axillary growth on main stem inhibited, a few leaves still alive near tips of stems, foliage reduced 90%, much curvature of stems, seed production reduced to less than 1% that of untreated plants.

Setaria lutescens Hub. (yellow foxtail); twenty-five plants.—No visible effect.

Setaria viridis Beauv. (green foxtail); twenty-five plants.—No visible effect.

Stellaria media Cyrill. (chickweed); more than 100 plants.—Within 5 days curvature of stems and severe browning of leaves, flowers arrested in development; at 13 days 90% of plants dead.

Taraxacum officinale Weber. (dandelion); three hundred plants.—Within 3 hours strong curvature of center leaves; at 1 day center leaves twisted 180°; at 2 days base of leaves whitish, elongate, enlarged; at 10 days leaves chlorotic, flaccid at base, complete absence of new flower stalks, old flower stalks elongate, chlorotic, and twisted (fig. 5); at 13 days all plants dead; at 30 days all roots disorganized and disintegrated.

Trifolium pratense L. (red clover); twenty-five plants.—Within 13 days browning of leaves touched by spray, plants subsequently recovered.

Mixture of common weeds in bluegrass lawn

The selective response observed in treatments of field mixtures of weeds made on July 25, in which plants of broad-leaved plantain, buckhorn plantain, and dandelion were killed whereas several grasses—including yellow foxtail, green foxtail, quack-grass, and Kentucky bluegrass—were not affected, suggested applications to bluegrass lawns infested with weeds.

Experiment 1.—On August 21, applications were made to a bluegrass lawn infested with dandelion and broad-leaved and buckhorn plantain, applying the material as a warm water spray (110° F.) directed at forty to fifty individual plants here and there on the lawn.

Fig. 4.—Effect of foliage sprays on milkweed, showing hyponasty and bending of stem.

Treated plants of dandelion and buckhorn plantain responded typically within a few hours, and all were dead within 10 days. Plants of broad-leaved plantain were less affected, and many were not killed although in a weakened condition. There was no visible injury to the grass.

Experiment 2.—On August 26, a carefully measured area 10 × 10 feet was selected in the center of a bluegrass lawn. The area contained 158 plants of buckhorn plantain, forty-five of dande-

FIG. 5.—Effect of foliage sprays on buckhorn plantain and dandelion, showing curvature, twisting, elongation of leaf bases, and arrest in flower development. Above, buckhorn plantain; below, dandelion.

lion, nine of round-leaved mallow (*Malva rotundifolia* L.), and a scattering of white clover (*Trifolium repens* L.). The spray was applied generously at a temperature of about 100° F. Six quarts of spray were used for the area. The weather was clear and sunny, and the air temperature was 70° F.

Within 2 days the plantain, dandelion, and mallow showed typical curvatures and chlorosis, and the main roots appeared water-soaked and discolored. The leaves of the white clover plants were curved downward. Within 13 days all plants of dandelion and plantain were dry and either completely dead or dying, the stems of mallow had proliferated and split, and the growth of white clover was severely checked. By September 25, 30 days after treatment, all plantain, dandelion, and mallow plants were dead, the tops being dried up and the roots disorganized. By October 17 not a single plant of these species remained alive, the only traces of any of them being a few brown vascular strands and fibers. Most of the white clover was dead, and that which was not entirely so was killed back to the main stolons and making feeble new growth, in contrast to the vigorous growth of clover in immediately adjacent unsprayed areas.

The bluegrass showed no evidences of injury that could be detected at any time. On the contrary, within 10 days of treatment the square of treated lawn was darker green in color and more vigorous than the rest of the lawn. On October 17, 52 days after treatment, the treated area was entirely free of dominant plant material other than grass and was sharply defined from the surrounding lighter green and less vigorous lawn area infested with plantain, dandelion, white clover, and mallow.

Woody plants

A few woody plants were sprayed at a concentration of 1000 p.p.m. during late July and early August, when they were relatively inactive. The results are fragmentary but are presented because of the contrast with herbaceous material. They do not preclude the possibility that higher concentrations and different times and methods of application might not produce different effects.

Malus domestica Borkh. (common apple); ten plants.—Minor curvature of tips and slight chlorosis of leaves near tips.

Rhus toxicodendron L. (poison ivy); 200 plants.—Within 1 day leaves drooping, new growth chlorotic; at 7 days leaves becoming more chlorotic; at 14 days slight recovery; at 36 days climbing plants defoliated; at 66 days all plants prematurely defoliated; at 76 days 50% of above-ground parts killed.

Rubus strigosus Michx. (red raspberry); twenty plants.—Curvature 3–6 inches back from tips, petioles re-curved, leaves folded upward along midrib, growth of tips arrested, stiff.

Rubus flagellaris Willd. (dewberry); twenty plants.—Within 1 day curvature 3–6 inches back from tips, leaves dry and brittle; at 75 days tips which were formerly curved killed back 4 inches.

Vitis vulpina L. (frost grape); ten plants.—Within 7 days curvature and browning of tips, resulting in killing of terminals back 4 inches.

II. Results with application as an aerosol

It was suggested in a previous paper (5) that perhaps the herbicidal effectiveness of 2,4-dichlorophenoxyacetic acid could be increased by applying it as an aerosol. This method has been used re-

cently for growth-regulating substances and is more fully described elsewhere (6). It is more nearly comparable to fumigation than to spraying.

An aerosol was prepared with 2% dichlorophenoxyacetic acid in 10% motor oil SAE 30 and 88% dimethyl ether. On September 9 it was applied from a 1-quart dispensing cylinder to two rows of apple nursery stock infested with bindweed and occasional plants of pigweed and purslane. The nozzle of the cylinder was directed downward at arm length and kept open while the operator walked rapidly down the row. The ground area covered was about 60 square yards, and half a pound of aerosol was used. No attempt was made to conserve material, so that no accurate data were obtained as to just how small an amount might have been equally effective. The temperature preceding the application was 55° F. Because of a brisk wind blowing, the aerosol drifted considerably.

In another area the material was applied horizontally about 2 feet from the ground with the small nozzle removed and discharged in a broad fanlike sweep 10 feet wide and 30 feet long.

Sixteen hours after treatment the plants of bindweed, purslane, and pigweed under both methods of application showed typical yet violent curvature, together with slight chlorosis. Plants were affected 30 feet to one side of the treated areas and beyond the point where any visible drifting of the material had occurred. Aerosol treatment can be more or less confined by the type of nozzle used, and no attempt was made in these treatments for spot application. Nevertheless, the effect of the material over such a broad area, and in what must have been very small concentrations, is of interest.

Five days after treatment many of the plants were dead, and 10 days after treatment all were dead. The plants treated by downward application at arm length were especially severely affected, probably owing to their receiving a higher concentration at close range than was the case with plants treated by a broad horizontal sweep.

The aerosol treatment was the most rapid and most effective treatment of any tested. The action is all the more striking since the air temperatures (55° F.) were lower than when the most effective liquid spray treatments were made (65°–90° F.) and in view of the fact that liquid-spray treatments at low temperatures (55° F.) were more slowly effective.

III. Residual effect in soil

To determine the residual effect of 2,-4-dichlorophenoxyacetic acid in the soil following applications as a herbicide, two treated areas were seeded to several cereal, lawn, and pasture plants.

EXPERIMENT 1.—One of the areas was the field mixture of common weeds which had been treated on August 21 with a light spraying at 1000 p.p.m. Vegetation was sparse at the time of treatment, and there is every likelihood that some material was applied directly to the soil surface, in addition to that which may have run off the foliage of sprayed plants. It was this area which had been seeded to white sweet clover just prior to the treatment and in which all young seedlings were killed as they emerged.

Plots 2 feet square were planted with cereals, grasses, and pasture plants on October 18 (58 days after treatment) under conditions favorable to germination. For comparison, similar plots were seeded on an untreated area immediately adjacent. By November 9, 22 days

after seeding, an excellent stand was secured of all species. No evidences of curvature or distortion were observed.

EXPERIMENT 2.—The second area was the lawn plot (10 × 10 feet) which had been sprayed with 6 quarts of material on August 25, and on which the killing of plantain, dandelion, and mallow had left bare spots. On October 18 (54 days after treatment) the bare spots were lightly seeded with Kentucky bluegrass. These, too, germinated and were not visibly affected.

Discussion

Many herbicides depend for their effectiveness on their caustic action, whereas the effectiveness of 2,4-dichlorophenoxyacetic acid depends on its conduction throughout the plant, the growth responses induced, and the destruction of specific tissue systems. Not only is the material selective for different species of plants, but it is also selective in the type of response which it brings about in different plants and different parts affected. Thus, the flowers and terminal growing points of above-ground parts of some plants may be arrested (as bindweed), or killed (as dewberry and grape). Some may become chlorotic (as poison ivy). Some may show epinasty (as purslane), and others may show hyponasty (as milkweed). The progressive curvature downward from the tips and the stiffening, dying, and splitting of above-ground stems is typical of pigweed, while the wilted appearance of the foliage of sow thistle and bindweed, and the proliferation, splitting, discoloration, and water-soaked appearance of the below-ground points are also typical.

The method of killing is of special significance when applied to such plants as bindweed and sow thistle, which regenerate not only from seed but also from shoots arising from underground stems and roots. BEAL (2) has shown that "pronounced morphological responses are often induced or incited at considerable distance from the point of application," and he has designated this response as "telemorphic." This is apparently the case with bindweed. How far the material travels may play a large part in its effectiveness as a herbicide.

2,4-Dichlorophenoxyacetic acid is especially effective against plants when in the meristematic condition. Actively growing bindweed and sow thistle are highly sensitive to this acid; and when thus stimulated, the increased activity must result in depletion of reserves to some degree. This depletion has been shown to be an important consideration in weed control (1, 4).

The apparent lack of response of the grasses at the time and concentrations used suggests the possibility of controlling undesired plants in lawns and turfs. There is also the suggestion from the reseeding treatments that light surface applications to cultivated land sometime prior to seeding might destroy some undesired weeds in young stages and yet not affect germination of the crop sown at a later date. A detailed study of the mechanism of action, responses to different concentrations, and effect of applications at different times and by different methods should yield profitable results.

Summary

1. Experiments were conducted in the immediate vicinity of Geneva, New York, between July 14 and October 14, 1944, using 2,4-dichlorophenoxyacetic acid as a herbicide applied as a water spray at a concentration of 1000 p.p.m. in Carbowax 1500 at the rate of one part acid to five parts Carbowax.

2. Applications to bindweed in rows of nursery stock on July 14 and July 31 resulted in drying out and killing of above-ground parts. Below-ground parts proliferated, became spongy and water-soaked, and decayed to a depth of at least 14 inches. Applied in warm water (110° F.), killing was more uniform and more rapid. In relatively cool weather (45°–75° F.) the response was much slower, and complete killing occurred only after 3–4 weeks. Bindweed was killed by immersing the tips in vials of the solution, and they were also killed when the foliage was sprayed exclusive of the tips.

3. Applications to sow thistle growing in a garden plot resulted in lighter green color and wilted appearance within 24 hours. The bases of the leaves became much enlarged and flattened, and the roots increased 50–300% in diameter and became soft and spongy. All plants were dead within 2 weeks.

4. Applications were made to field mixtures of common weeds. There was no visible effect upon quack-grass, Kentucky bluegrass, yellow foxtail, green foxtail, wild oats, large crab-grass, small crab-grass, barnyard grass, and goose-grass. Bindweed, narrow-leaved plantain, dandelion, round-leaved mallow, lambs-quarters, and ragweed were killed following varying formative responses. Pigweed, milkweed, tomato, broad-leaved plantain, Pennsylvania smartweed, purslane, chickweed, and red clover showed varying responses, including epinastic curvatures, splitting of stems, swelling of stems and roots, browning of leaves, stems, and roots, chlorosis of leaves and stems, elongation, chlorosis and enlargement of petioles, and killing of some plants.

5. Germinating seedlings of white sweet clover emerging 3–7 days after the soil surface had been sprayed were completely killed.

6. Applications were made to a bluegrass lawn infested with dandelion, buckhorn plantain, round-leaved mallow, and white clover. All plants of dandelion, plantain, and mallow were dead and disintegrated within 30 days; most of the white clover was dead, and that which was not dead was killed back to the main stolons; and the bluegrass became dark green in color but otherwise not visibly affected.

7. A few woody plants were treated. The apple showed minor curvature and mild chlorosis near the tips; poison ivy showed chlorosis and arrested development and 50% killing of above-ground parts; red raspberry, formative effects 3–6 inches back from the tips; and dewberry and grape showed formative effects and killing 4 inches back from the tips.

8. Applications by the aerosol method, using 2% 2,4-dichlorophenoxyacetic acid in 10% motor oil SAE 30 and 88% dimethyl ether, proved effective against bindweed, pigweed, and purslane.

9. The residual effect of the acid in the soil after application as a herbicide was tested. Twelve species of cereal, lawn, and pasture plants were sown on an area which had been sprayed 2 months earlier. The seeds germinated and the young plants showed no curvatures or formative effects.

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Plants and Vitamins. By W. H. SCHOPFER. Translated by N. L. NOECKER. Waltham, Mass.: Chronica Botanica Co., 1943. Pp. 293. Illustrated. $4.75.

A great deal of the attention of biologists has been and is absorbed in the study of growth, and with good reason, for it is one of the most significant characteristics of living matter. When it is found, therefore, that the growth of any organism depends upon the presence of a minute quantity of a single substance, the discovery naturally affects the thinking of all those who are interested in this most important process. SCHOPFER's demonstration of the essential nature of thiamine for the development of the bread mold was a discovery of that type, and its results have profoundly influenced many researches. As the ripples from a stone dropped in a pool spread to a distant shore, so the discovery that some plants suffer from vitamin deficiencies has had far reaching effects.

SCHOPFER's discovery is interesting in another respect. It shows how the investigation of a phenomenon which on the surface appears trivial may have very considerable ultimate results. SCHOPFER found the essential nature of thiamine for *Phycomyces* by searching for an explanation for the failure of this fungus to grow when one sample of maltose was used as a source of carbon and its ability to flourish when another sample of maltose was supplied. The answer, as he eventually learned, was the presence of thiamine (or its intermediates) in the one sample and its absence in the other. These preliminary remarks will explain in part why SCHOPFER should have written this book and why his text is of interest to botanists and to all others concerned with the problem of growth.

The book contains twenty-four chapters, with a conclusion, author index, general index, and illustrations. It is divided into three parts as follows. Part I: Synthesis of vitamins in plants; Auxotrophic plants; Research methods. Part II: Vitamins in relation to plants unable to synthesize them; Growth factors for microörganisms. Part III: General problems involving vitamins. Each chapter is followed by a bibliography. The text is clearly written, and the author has not hesitated to include chemical formulae and chemical discussion when necessary to elucidate the topic under consideration.

Much has been added to the field since the text was completed, but it stands as a source book which should be available to everyone who works with plants, both phanerogams and cryptogams.—W. J. ROBBINS.

Thomas Jefferson and the Scientific Trends of His Time. By CHARLES A. BROWNE. Waltham, Mass.: Chronica Botanica Co., 1944. Pp. 423. $1.25.

This is a brief presentation of some of the salient facts concerning THOMAS JEFFERSON'S versatility, including some of his ventures and speculations in the fields of science. His statement that "it is always better to have no ideas than false ones; to believe nothing rather than to believe what is wrong," seems to be part of the current preachments of present-day educators as well.—E. J. KRAUS.

Aquatic Plants of the United States. By WALTER C. MUENSCHER. Ithaca, N. Y.: Comstock Publishing Co., 1944. Pp. 374. Illustrated. $5.00.

This contribution helps greatly toward filling the gap in available information on aquatic plants. The material, which covers the entire United States, is presented in a manner so clear that interested laymen having a general knowledge of botanical terminology and trained botanists alike will find it most useful.

Diagnostic keys to families, genera, and species, simple clean-cut critical drawings, and distributional maps all aid in making identification more simple and direct.—E. J. KRAUS.

The Naturalist's Lexicon. By ROBERT S. WOODS. Pasadena, Calif.: Abbey Garden Press, 1944. Pp. 282. $2.75.

To biologists interested in the English equivalents of some technical names and phrases, or in inventing new technical terms, this brief work should prove useful. Most of the space of the book is devoted to a Classical-English lexicon, followed by a much briefer English-Classical supplement, in which may be found a limited list of nouns, adjectives, verbs, and prefixes relating to both animals and plants.—E. J. KRAUS.

The Aquatic Oomycetes of Wisconsin: Part I. By FREDERICK TAYLOR WOLF. Madison, Wis.: University of Wisconsin Press, 1944. Pp. 64. $1.50.

This brief volume contains an assemblage of the results of the investigations of the several previous collectors' reports on the aquatic Oomycetes of Wisconsin, combined with the findings from several thousand collections of water and soil samples made by the author during a period of more than 2 years and cultured for the presence of aquatic fungi. Adequate descriptions of the forms, records of their collections, and keys for their identification have been included. Slightly more than fifty species are listed. —J. M. BEAL.

A Source Book of Agricultural Chemistry. By CHARLES A. BROWNE. Waltham, Mass.: Chronica Botanica Co.; New York City: G. E. Stechert Co. $5.00.

The author gives as his definition of agricultural chemistry, "that branch of applied science which deals with the chemical composition and mutual chemical relations of soils, fertilizers, crops, and farm animals in so far as they concern the production upon the farm of agricultural supplies," and states further that "The mutual relationships of crops to animals and of animals to crops constitute, however, such an important part of agricultural science that they must be considered in any comprehensive treatise of agricultural chemistry."

With this as a basis for selection of material, a history of the subject from the early Greek nature philosophers (640–430 B.C.) through most of the experimentally productive activity of LIEBIG—terminating with 1852—is presented. Students of plant physiology will find much that is familiar, since most of the subject matter deals with plants in relation to their environment. Illustrative material and quotations are presented to much greater length and with far more detail than is usually encountered in most botanical text-books now available. Comments by the author are to the point and often helpfully critical. The book is a substantial addition to the literature treating of botanical history.—E. J. KRAUS.